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(54) Title: PANCREATIC POLYPEPTIDE FAMILY MOTIFS AND POLYPEPTIDES COMPRISING THE SAME

(57) Abstract: The present invention relates to novel Pancreatic Polypeptide Family ("PPF") polypeptides. The PPF polypeptides of the invention generally include at least two PPF motif, have at least 50 % sequence identity to PYY (3-36) over its length and will generally retain, at least in part, a biological activity of a PP, PYY or NPY Preferred PPF polypeptides of the invention are those having a potency in one of the assays described herein (preferably food intake, gastric emptying, pancreatic secretion, or weight reduction assays) which is greater than the potency of PP, NPY, PYY, or PYY(3-36) in that same assay. In one aspect, the PPF polypeptides of the invention include novel PYY analog polypeptides. In another aspect, the PPF polypeptides of the invention include PPF chimeric polypeptides including a fragment of a PP family polypeptide linked to a second PP family polypeptide, wherein each of the first and second fragments includes a PPF motif. Methods of using the PPF polypeptides of the invention, and pharmaceutical compositions including the PPF polypeptides of the invention are also disclosed.

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PANCREATIC POLYPEPTIDE FAMILY MOTIFS AND POLYPEPTIDES COMPRISING THE SAME

FIELD OF THE INVENTION

The present invention relates to peptide chemistry, and more particularly to pancreatic polypeptide family ("PPF") polypeptides.

BACKGROUND OF THE INVENTION

A number of related hormones make up the pancreatic polypeptide family ("PPF"). Pancreatic polypeptide ("PP") was discovered as a contaminant of insulin extracts and was named by its organ of origin rather than functional importance (Kimmel *et al, Endocrinology* 83: 1323-30 (1968)). PP is a 36-amino acid peptide (SEQ ID NO: 1) containing distinctive structural motifs. A related peptide was subsequently discovered in extracts of intestine and named Peptide YY ("PYY") (SEQ ID NO: 2) because of the N- and C-terminal tyrosines (Tatemoto, *Proc. Natl. Acad. ScL USA 19:* 2514-8 (1982)). A third related peptide was later found in extracts of brain and named Neuropeptide Y ("NPY") (SEQ ID NO: 4) (Tatemoto, *Proc. Natl. Acad. Sci. USA* 79: 5485-9 (1982); Tatemoto *et al, Nature* 296: 659-60 (1982)).

These three related peptides have been reported to exert various biological effects. Effects of PP include inhibition of pancreatic secretion and relaxation of the gallbladder. Centrally administered PP produces modest increases in feeding that may be mediated by receptors localized to the hypothalamus and brainstem (reviewed in Gehlert, *Proc. Soc. Exp. Biol. Med.* 218: 7-22 (1998)).

Release of PYY (SEQ ID NO: 2) occurs following a meal. An alternate molecular form of PYY is PYY(3-36) (SEQ ID NO: 3) (Eberlein *et al, Peptides* 10: 797-803 (1989); Grandt *et al, Regul. Pept.* 51: 151-9 (1994)). This fragment constitutes approximately 40% of total PYY-like immunoreactivity in human and canine intestinal extracts and about 36% of total plasma PYY immunoreactivity in a fasting state to slightly over 50% following a meal. It is apparently a dipeptidyl peptidase-IV (DPP4) cleavage product of PYY. PYY(3-36) is reportedly a selective ligand at the Y2 and Y5 receptors, which appear pharmacologically unique in preferring N-terminally truncated *(i.e.,* C-terminal fragments of) NPY analogs. Peripheral

administration of PYY reportedly reduces gastric acid secretion, gastric motility, exocrine pancreatic secretion (Yoshinaga *et al, Am. J. Physiol.* 263: G695-701 (1992); Guan *et al, Endocrinology* 128: 911-6 (1991); Pappas *et al, Gastroenterology* 91: 1386-9 (1986)), gallbladder contraction and intestinal motility (Savage *et al, Gut* 28: 166-70 (1987)). The effects of central injection of PYY on gastric emptying, gastric motility and gastric acid secretion, as seen after direct injection in or around the hindbrain/brainstem (Chen and Rogers, *Am. J. Physiol.* 269: R787-92 (1995); Chen *et al, Regul Pept.* 61: 95-98 (1996); Yang and Tache, Am. *J. Physiol.* 268: G943-8 (1995); Chen *et al, Neurogastroenterol Motil.* 9: 109-16 (1997)), may differ from those effects observed after peripheral injection. For example, centrally administered PYY had some effects opposite to those described herein for peripherally injected PYY(3-36) in that gastric acid secretion was stimulated, not inhibited. Gastric motility was suppressed only in conjunction with TRH stimulation, but not when administered alone, and was indeed stimulatory at higher doses through presumed interaction with PP receptors. PYY has been shown to stimulate food and water intake after central administration (Morley *et al, Brain Res.* 341: 200-3 (1985); Corp *et al, Am. J. Physiol* 259: R317-23 (1990)).

Likewise, one of the earliest reported central effects of NPY (SEQ ID NO: 4) was to increase food intake, particularly in the hypothalamus (Stanley *et al, Peptides* 6: 1205-1 1 (1985)). PYY and PP are reported to mimic these effects, and PYY is more potent or as potent as NPY (Morley *et al, Brain Res.* 341: 200-3 (1985); Kanatani *et al, Endocrinology* 141: 1011-6 (2000); Nakajima *et al, J. Pharmacol. Exp. Ther.* 268: 1010-4 (1994)). Several groups found the magnitude of NPY-induced feeding to be higher than that induced by any pharmacological agent previously tested, and also extremely long-lasting. NPY-induced stimulation of feeding has been reproduced in a number of species. Among the three basic macronutrients (fat, protein, and carbohydrate), the intake of carbohydrates was preferentially stimulated. No tolerance was seen towards the orexigenic effect of NPY, and when administration of the peptide was repeated over 10 days, a marked increase in the rate of weight gain was observed. Following starvation, the concentration of NPY in the hypothalamic PVN increased with time, and returned rapidly to control levels following food ingestion.

Pharmacological studies and cloning efforts have revealed a number of seven transmembrane receptors for the PP family of peptides, and these receptors have been assigned the names Y1 through Y6 (and a putative PYY-preferring receptor *YT)*. Typical signaling responses of these receptors are similar to those of other G;/Go-coupled receptors, namely inhibition of adenylate cyclase. Even with fairly low sequence homology among receptors, it is apparent that there is a clustering of amino acid sequence similarity between Yl, Y4 and Y6 receptors, while Y2 and Y5 define other families. Other binding sites have been identified by the rank order of potency of various peptides. The NPY-preferring receptor, which has not been cloned, has been termed Y3, and PYY-preferring receptors have also been shown to exist (putative *Yl)* (reviewed in Michel *et al, Pharmacol. Rev.* 50:143-50 (1998); Gehlert, *Proc. Soc. Exp. Biol. Med.* 218: 7-22 (1998)).

The Y5 and Y1 receptors have been suggested as the primary mediators of the food intake response (Marsh *et al, Nat. Med.* 4: 718-21 (1998); Kanatani *et al, Endocrinology* 141: 1011-6 (2000)). The prevalent idea has been that endogenous NPY, via these receptors, increases feeding behavior. Proposed therapies for obesity have invariably been directed toward antagonism of NPY receptors, while therapies for treating anorexia have been directed toward agonists of this ligand family *{see, e.g.,* U.S. Patent Nos. 5,939,462; 6,013,622; and 4,891,357). In general, PYY and NPY are reported to be equipotent and equally effective in all Yl, Y5 (and Y2) receptor assays studied (Gehlert, *Proc. Soc. Exp. Biol Med.* 218: 7-22 (1998)).

Pharmacologically, the Y2 receptor is distinguished from Y1 by exhibiting affinity for C-terminal fragments of neuropeptide Y. The Y2 receptor is most often differentiated by the affinity of neuropeptide Y(13-36), although the 3-36 fragment of neuropeptide Y and peptide YY provided improved affinity and selectivity (see Dumont et a., *Soc. for Neurosci. Abstracts* 19:726 (1993)). Signal transmission through both the Y1 and Y2 receptors are coupled to the inhibition of adenylate cyclase. Binding to the Y2 receptor was also found to reduce the intracellular levels of calcium in the synapse by selective inhibition of N-type calcium channels. In addition, the Y2 receptor, like the Y1 receptors, exhibits differential coupling to second messengers *(see* U.S. Patent No. 6,355,478). Y2 receptors are found in a variety of brain regions, including the hippocampus, substantia nigra-lateralis, thalamus, hypothalamus, and brainstem. The

human, murine, monkey and rat Y2 receptors have been cloned *(e.g., see* U.S. Patent No. 6,420,352 and U.S. Patent No. 6,355,478).

The main characteristic of putative Y3 receptors is that they recognize NPY, while PYY is at least an order of magnitude less potent. The Y3 receptor represents the only binding site/receptor that shows a preference for NPY.

There is an additional binding site/receptor which shows preference for PYYs, termed PYY-preferring receptor, which is referred to herein as the Y7 receptor(s). Different rank orders of binding to this receptor, or class of receptors, have been reported, suggesting that there may be more than one receptor in this family. In most cases it has been applied to describe a receptor where PYY was three to five times more potent than NPY. The International Union of Pharmacology recommendations for the nomenclature of NPY, PYY and PP receptors are that the term PYY-preferring receptor is not used unless a potency difference of at least twenty-fold between PYY and NPY is observed (Michel *et al, Pharmacol. Rev.* 50: 143-50 (1998)). However, for purposes of this disclosure, reference to the Y7 receptor or pharmacology of a PYY-preferring receptor means a receptor having any degree of preference for PYY over NPY.

Obesity and its associated disorders are common and very serious public health problems in the United States and throughout the world. Upper body obesity is the strongest risk factor known for type 2 diabetes mellitus, and is a strong risk factor for cardiovascular disease. Obesity is a recognized risk factor for hypertension, atherosclerosis, congestive heart failure, stroke, gallbladder disease, osteoarthritis, sleep apnea, reproductive disorders such as polycystic ovarian syndrome, cancers of the breast, prostate, and colon, and increased incidence of complications of general anesthesia *(see, e.g.,* Kopelman, *Nature* 404: 635-43 (2000)). It reduces life-span and carries a serious risk of co-morbidities above, as well disorders such as infections, varicose veins, acanthosis nigricans, eczema, exercise intolerance, insulin resistance, hypertension hypercholesterolemia, cholelithiasis, orthopedic injury, and thromboembolic disease (Rissanen *et al, Br. Med. J.* 301: 835-7 (1990)). Obesity is also a risk factor for the group of conditions called insulin resistance syndrome, or "Syndrome X." Recent estimate for the medical cost of obesity and associated disorders is $150 billion worldwide. The pathogenesis of obesity is believed to be

multifactorial but the basic problem is that in obese subjects nutrient availability and energy expenditure do not come into balance until there is excess adipose tissue. Obesity is currently a poorly treatable, chronic, essentially intractable metabolic disorder. A therapeutic drug useful in weight reduction of obese persons could have a profound beneficial effect on their health.

There remains a need to develop further PYY analog polypeptides. Accordingly, it is an object of the present invention to provide such PYY analog polypeptides and methods for producing and using them.

All documents referred to herein are incorporated by reference into the present application as though fully set forth herein.

SUMMARY OF THE INVENTION

The present invention relates generally to pancreatic polypeptide family ("PPF") polypeptides having at least 50% sequence identity to PYY(3-36) over the entire length of PYY(3-36), and also comprise at least two PPF motifs including at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif. Additional PPF motifs of the invention may correspond to any motif of any of the PP family polypeptides, including PP, PYY and NPY. In certain embodiments, the PPF polypeptides do not include unnatural amino acids. In other embodiments, the PPF polypeptides do not include known naturally occurring species variants.

In one aspect, the PPF polypeptides of the invention include PYY analog polypeptides. In yet another aspect of the invention, the PPF polypeptides of the invention include PPF chimeric polypeptides comprising a fragment of a PP, PYY or NPY polypeptide covalently linked to at least one additional fragment of a PP, PYY or NPY polypeptide, wherein each PP, PYY or NPY fragment includes a PPF motif. Such PPF analog polypeptides and PPF chimeric polypeptides of the invention will exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). In certain embodiments, desirable PPF chimeric polypeptides include an N-terminal PP fragment in combination with a C-terminal PYY fragment. In other embodiments, PPF chimeric polypeptides include an N-terminal PP fragment in combination with a C-terminal NPY fragment. In other embodiments, PPF chimeric polypeptides include an N-terminal PYY fragment and a C-terminal PP or NPY

fragment. In other embodiments, PPF chimeric polypeptides include an N-terminal NPY in combination with a C-terminal PYY or PP. hi other embodiments, PPF chimeric polypeptides may not include an N-terminal PP fragment in combination with a C-terminal NPY fragment. In still other embodiments, PPF chimeric polypeptides may not include an N-terminal NPY fragment with a C-terminal PYY fragment.

In another aspect of the invention, methods for treating or preventing obesity are provided, wherein the method comprises administering a therapeutically or prophylactically effective amount of a PPF polypeptide of the invention to a subject in need thereof. In a preferred embodiment, the subject is an obese or overweight subject. While "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese." Subjects who are insulin resistant, glucose intolerant, or have any form of diabetes mellitus (*e.g.,* type 1, 2 or gestational diabetes) can benefit from this method.

In yet another aspect of the invention, compounds of the invention can be used for methods of reducing food intake, reducing nutrient availability, causing weight loss, affecting body composition, altering body energy content or energy expenditure and improving lipid profile (including reducing LDL cholesterol and triglyceride levels and/or changing HDL cholesterol levels). Thus, in certain embodiments, the methods of the invention are useful for treating or preventing conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of a PPF polypeptide of the invention. Such conditions and disorders include, but are not limited to, hypertension, dyslipidemia, cardiovascular disease, eating disorders, insulin-resistance, obesity, diabetes mellitus of any kind, including Type I, Type II, and gestational diabetes. Compounds of the invention may also be useful in treating or preventing other conditions associated with obesity including stroke, cancer (*e.g,.* endometrial, breast, prostate, and colon cancer), gallbladder disease, sleep apnea, reduced fertility, and osteoarthritis, (*see* Lyznicki *et al, Am. Fam. Phys.* 63:2185, 2001).

Compounds of the invention may also be useful for potentiating, inducing, enhancing or restoring glucose responsivity in pancreatic islets or cells. These actions may also be used to treat or prevent conditions associated with metabolic disorders such as those described above and in U.S. patent application no. US20040228846.

In addition to the amelioration of hypertension in subjects in need thereof as a result of reduced food intake, weight loss, and treating obesity, compounds of the invention may be used to treat or prevent hypotension.

Compounds of the invention may also be useful in the treatment or prevention of any number of gastrointestinal disorders that are associated with excess intestinal electrolytes and water secretion as well as decreased absorption, *e.g.,* infectious *(e.g.,* viral or bacterial) diarrhea, inflammatory diarrhea, short bowel syndrome, or the diarrhea which typically occurs following surgical procedure, e.g., ileostomy *(see e.g.,* Harrison's principles of Internal Medicine, McGraw Hill Inc., New York, 12th *ed).* Examples of infectious diarrhea include, without limitation, acute viral diarrhea, acute bacterial diarrhea *(e.g.,* salmonella, Campylobacter, and Clostridium) or diarrhea due to protozoal infections, or travellers' diarrhea *(e.g.,* Norwalk virus or rotavirus). Examples of inflammatory diarrhea include, without limitation, malabsorption syndrome, tropical spue, chronic pancreatitis, Crohn's disease, diarrhea, and irritable bowel syndrome. It has also been discovered that the peptides of the invention can be used to treat or prevent an emergency or life-threatening situation involving a gastrointestinal disorder, e.g., after surgery or due to cholera. Furthermore, the compounds of the invention can be used to treat intestinal dysfunction in patients with Acquired Immune Deficiency Syndrome (AIDS), especially during cachexia. The compounds of the invention may also be useful for inhibiting small intestinal fluid and electrolyte secretion, and augmenting nutrient transport, as well as increasing cell proliferation in the gastrointestinal tract, regulating lipolysis in, *e.g.,* adipase tissue and regulating blood flow in a mammal.

Compounds of the invention may also be useful for treating or preventing the above conditions by their gastrointestinal protective activity. Accordingly, compound of the invention may be used to treat gastrointestinal or muscosal damage. Exemplary types of damage include, but are not limited to, inflammatory bowel disease, bowel atrophy, conditions characterized by loss of bowel mucosa or bowel mucosal function, and

other conditions of the gastrointestinal tract, including those which may be brought about by exposure to cytotoxic agents, radiation, toxicity, infection and/or injury. Moreover, these compounds of the invention may be combined with analgesics, anti-inflammatory agents, growth hormone, heparin, or any other therapies that may be used to treat inflammatory bowel disease or other conditions listed above.

Moreover, compounds of the invention are useful in treating or preventing diseases and disorders that can be alleviated or ameliorated by their anti-secretory properties. Such anti-secretory properties include inhibition of gastric and/or pancreatic secretions and can be useful in the treatment or prevention of diseases and disorders including gastritis, pancreatitis, Barrett's esophagus, and Gastroesophageal Reflux Disease. These diseases may also be treated or prevented by the gastrointestinal protective functions of compounds of the invention.

Compounds of the invention may also be useful for reducing aluminum concentrations in the central nervous system of a subject to treat or prevent a disease or condition associated with abnormal aluminum concentrations (*e.g.,* a patient afflicted with Alzheimer's disease or at risk for developing Alzheimer's disease, dialysis dementia, or increased aluminum levels due to occupational exposure).

The present invention also relates to pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of at least one PPF polypeptide of the invention, or a pharmaceutically acceptable salt thereof, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in the delivery of the PPF polypeptides.

These and other aspects of the invention will be more clearly understood with reference to the following preferred embodiments and detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the activity of certain PPF polypeptides of the invention in a food intake assay.

Figure 2 demonstrates the activity of additional PPF polypeptides of the invention in a food intake assay.

Figure 3 demonstrates the activity of yet additional PPF polypeptides of the invention in a food intake assays.

Figure 4 demonstrates the activity of yet additional PPF polypeptides of the invention in a food intake assay.

Figure 5 demonstrates the activity of certain PPF polypeptides of the invention in the **DIO** mouse model.

Figure 6 demonstrates the activity of additional PPF polypeptides of the invention in the **DIO** mouse model.

Figure 7 shows weight gain in rats.

Figure 8 demonstrates the activity of **a** PPF polypeptide of the invention in a food intake assay and the **DIO** mouse model, as compared to PYY(3-36).

Figures 9A-9D demonstrate the effect of PPF polypeptides of the invention on heart rate and blood pressure, as compared to PYY and PYY(3-36).

Figure 10 demonstrates the activity of PPF polypeptides of the invention on gastric acid secretion.

Figure 11 demonstrates the activity of PPF polypeptides of the invention on gastric acid secretion.

Figure 12 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 13 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 14 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 15 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 16 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 17 demonstrates the activity of PPF polypeptides of the invention on gastric emptying.

Figure 18 demonstrates the activity of PPF polypeptides of the invention on gallbladder emptying.

Figure 19 demonstrates the activity of PPF polypeptides of the invention on gallbladder emptying.

Figure 20 demonstrates the activity of PPF polypeptides of the invention on gastric mucosal protection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to pancreatic polypeptide family ("PPF") polypeptides having at least 50% sequence identity to PYY(3-36) over the entire length of PYY(3-36). The PPF polypeptides of the invention also comprise at least two PPF motifs including at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif. Additional PPF motifs of the invention may correspond to a motif of any of the PP family polypeptides, including PP, PYY and NPY, for example the type II β-turn region motif of PYY, or the α-helical motif at the C-terminal end of PYY. In certain embodiments, the PPF polypeptides of the invention may not include any unnatural amino acids.

The present invention also relates to PPF polypeptides useful in the treatment and prevention of metabolic conditions and disorders. In a preferred embodiment, the PPF polypeptides of the invention may have comparable or higher potency in the treatment and/or prevention of metabolic conditions and disorders, as compared to native human PP, PYY, PYY(3-36) or NPY. Alternatively, preferred PPF polypeptides of the invention may exhibit less potency but may possess other desirable features such as improved ease of manufacture, stability, and/or ease of formulation, as compared to PP, PYY, PYY(3-36), or NPY.

In a preferred embodiment, and without intending to be limited by theory, it is believed that the peripheral administration of the novel PPF polypeptides of the invention to a subject reduces nutrient availability, and thus is useful in the treatment and prevention of obesity and related metabolic conditions or disorders. As such, the present invention provides PPF polypeptide compositions and methods of using them to reduce nutrient availability in a subject in need thereof for treating and preventing metabolic conditions or disorders that may benefit from a reduction in nutrient

availability. These methods may be useful in the treatment of, for example, obesity, diabetes, including but not limited to type 2 or non-insulin dependent diabetes, eating disorders, insulin-resistance syndrome, and cardiovascular disease.

The section headings are used herein for organizational purposes only, and are not to be construed as in any way limiting the subject matter described.

PPF Polypeptides of the Invention and PPF Motifs

As discussed above, the present invention relates at least in part to novel PPF polypeptides comprising at least two PPF motifs, wherein the at least two PPF motifs include at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif. The PPF polypeptides of the invention will also exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). The polypeptides of the present invention will preferably retain, at least in part, a biological activity of native human PP, PYY or NPY, *e.g.*, the polypeptides of the present invention will generally be PP, PYY and/or NPY agonists or antagonists. In a preferred embodiment, the polypeptides of the present invention will exhibit biological activity in the treatment and prevention of metabolic conditions and disorders. Further, the PPF polypeptides of the invention may include internal linker compounds, may include chemical modifications at internal amino acid residues, or may be chemically modified at the N-terminal or C-terminal residue. In a preferred embodiment, the polypeptides of the invention include only natural L amino acid residues and/or modified natural L amino acid residues. Alternatively, in a preferred embodiment, the polypeptides of the invention do not include unnatural amino acid residues.

The PPF motifs of the invention may correspond to any motif of any of the native PP family polypeptides, including PP, PYY and NPY. A "PPF motif is generally a structural component, primary, secondary, or tertiary, of a native PP family polypeptide that is critical to biological activity, *i.e.*, biological activity is substantially decreased in the absence or disturbance of the motif. Preferred PPF motifs include the N-terminal polyproline type II motif of a native PP family polypeptide, the type II β-turn motif of native PP family polypeptide, the α-helical motif at the C-terminal end of native PP family polypeptide, and the C-terminal tail

motiff of native PP family polypeptide. More particularly, in the N-terminal polyproline region, amino acids corresponding to residues 5 and 8 of a native PP family polypeptide are generally conserved as a proline. The type II β-turn motif will generally include amino acids corresponding to residues 12-14 of a native PP family polypeptide. The α-helical motif can generally extend from amino acids corresponding to approximately residue 14 of a native PP family polypeptide to any point up to and including the C-terminal end, so long as the α-helical motif includes a sufficient number of amino acid residues such that an α-helical turn is formed in solution. The α-helical motif can also include amino acid substitutions, insertions and deletions to the native PP family sequence, so long as the α-helical turn is still formed in solution. The C-terminal tail motif generally includes amino acids corresponding to approximately the last 10 residues of a native PP family polypeptide, more preferably the last 7, 6, or 5 residues of a native PP family polypeptide, and more preferably amino acid residues 32-35.

In one embodiment, the PPF polypeptides of the invention do not include any unnatural amino acid resides, and further with the provisio that the PPF polypeptides of the invention do not include any native PPF polypeptides (*e.g.,* PP, NPY(l-36), NPY(3-36), PYY(I -36), PYY(3-36), NPY(2-36), NPY(4-36), PYY(2-36), PYY(4-36), PP(2-36), PP(3-36), or PP(4-36)). The PPF polypeptides of the invention also preferably do not include: TyT^{l}IiPP. Lys^{4}hPP, Asn^{7}hPP, Arg^{19}hPP, Tyr^{21}hPP, GIu^{21}IiPP, AIa^{23}IiPP, Gln^{23}hPP, GIn^{34}IiPP$_5$ Phe^{6}Arg^{19}hPP, Phe^{6}Tyr^{21}hPP, Phe^{6}Glu^{21}hPP, Phe^{6}Ala^{23}hPP, Phe^{6}Gln^{23}hPP, Pro^{13}Ala^{14}hPP, He^{31}GIn^{34}PP, Arg^{19}Tyr^{20}Tyr^{21}Ser^{22}Ala^{23}hPP, Lys^{4}Arg^{19}Tyr^{20}Tyr^{21}Ser^{22}Ala^{23}hPP, Lys^{4}Arg^{19}Tyr^{20}Tyr^{21}Ser^{22}Ala^{23}hPP(2-36), AIa^{l}NPY, Tyr'NPY, AIa^{2}NPY, LeU^{2}NPY, Phe^{2}NPY, HiS^{2}NPY, AIa^{3}NPY, AIa^{4}NPY, AIa^{6}NPY, Tyr^{7}pNPY, AIa^{7}NPY, AIa^{9}NPY, AIa^{10}NPY, AIa^{11}NPY, GIy^{12}NPY$_5$ AIa^{13}NPY, GIy^{14}NPY, AIa^{15}NPY$_5$ AIa^{16}NPY, AIa^{17}NPY, GIy^{18}NPY, AIa^{19}NPY, LyS^{19}NPY, AIa^{20}NPY, AIa^{21}NPY, AIa^{22}NPY$_5$ GIy^{23}NPY, AIa^{24}NPY$_5$ Trp^{24}pNPY$_5$ AIa^{25}NPY, LyS^{25}NPY, AIa^{26}NPY$_5$ AIa^{27}NPY, Phe^{27}NPY, AIa^{28}NPY, AIa^{29}NPY$_5$ GIn^{29}NPY, AIa^{30}NPY$_5$ Phe^{30}NPY, AIa^{31}NPY$_5$ Trp^{31}PNPY$_5$ AIa^{32}NPY$_5$ Trp^{32}NPY, AIa^{33}NPY, LyS^{33}NPY, AIa^{34}NPY$_5$ Pro^{34}NPY, LeU^{34}NPY$_3$AIa^{35}NPY, LyS^{35}NPY$_5$AIa^{36}NPY, Phe^{36}NPY$_5$HiS^{36}NPY, Glu^{4}Pro^{34}pNPY, Arg^{6}Pro^{34}pNPY, Phe^{6}Pro^{34}pNPY, Cys^{6}Pro^{34}pNPY$_5$Asn^{6}Pro^{34}pNPY$_5$Phe^{7}Pro^{34}pNPY,

Arg^{7}Pro^{34} pNPY, Cys^{7}Pro^{34} pNPY, Asp^{7}Pro^{34} pNPY, Phe^{8}Pro^{34} pNPY, Arg^{8}Pro^{34} pNPY, Cys^{8}Pro^{34} pNPY, Asp^{8}Pro^{34} pNPY, As$_{n}$^{8}PrO^{34}PM'Y, Pro^{u} Pro^{34}pNPY$_{5}$ Ser^{13}Pro^{14}pNPY, Trp$^{24'31}$pNPY, Ala^{31}Pr$_{0}$^{32}PNPY, Cys^{31}Pr$_{0}$^{34}PNPY, LeU^{31}Pr$_{0}$^{34}NPY, Phe^{32}Pro^{34}pNPY, Ala$^{21>25}$Pro^{34}pNPY, Pro^{11}TyT^{13}Pr$_{0}$^{14}PrO^{34}PNPY, Ahx(9-22)pNPY$_{5}$ Ahx(9-17)pNPY, des-AA(10-20)-Cys^{7,21}Pro^{34}-pNPY, des-AA(10-17)-pNPY, des-AA(10-17)-Cys$^{2'27}$-pNPY, des-AA(10-17)-Ala$^{7'21}$-pNPY, des-AA(10-17)-Cys^{7,21}-pNPY, des-AA(10-17)-Glu^{7}Lys^{21}-pNPY, des-AA(10-17)Cys$^{7>21}$Pro^{34}pNPY, des-AA(10-17)Glu^{7}Lys^{21}Pro^{34}pNPY, des-AA(10-17)Cys^{7,21}Leu^{31}Pro^{34}pNPY, des-AA(1 1-17)Cys$^{7'21}$Pro^{34}pNPY, Pr$_{0}$^{34}PYY, HiS^{34}PYY, Lys^{25}hPYY(5-36), Arg^{4}hPYY(4-36), Gln^{4}hPYY(4-36), Asn^{4}hPYY(4-36), Lys^{25}hPYY(4-36), Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Tyr^{1,36}pPYY, Pro^{13}Ala^{14}hPYY, Leu^{31}Pr$_{0}$^{34}PYY, FMS-PYY, FMS-PYY(3-36), Fmoc-PYY, Fmoc-PYY(3-36), FMS$_{2}$-PYY, FMS$_{2}$-PYY(3-36), Fm$_{0}$C$_{2}$-PYY, Fm$_{O}$c$_{2}$-PYY(3-36), hPP(l-7)-pNPY, hPP(l-17)-pNPY, hPP(19-23)-pNPY, hPP(19-23)-Pro^{34}pNPY, hPP(19-23)-His^{34}pNPY, rPP(19-23)-pNPY, rPP(19-23)-Pro^{34}pNPY, rPP(19-23)-His^{34}pNPY, hPP(l-7)-pNPY, hPP(l-17)-pNPY, hPP(l-17)-His ^{34}pNPY, pNPY(l-7)-hPP, pNPY(l-7, 19-23)-hPP, cPP(l-7)-pNPY(19-23)-hPP, cPP(l-7)-NPY(19-23)-His ^{34}hPP, hPP(l-17)-His ^{34}pNPY, hPP(19-23)-pNPY, hPP(19-23)-Pr$_{O}$^{34}pNPY, hPP(19-23)-His^{34}pNPY, rPP(19-23)-pNPY, rPP(19-23)-Pro^{34}pNPY, rPP(19-23)-His^{34}pNPY, pNPY(l-7)-hPP, $_{P}$NPY(19-23)-hPP, pNPY(19-23)-Gln^{34}hPP, pNPY(19-23)-His^{34}hPP, pNPY(19-23)-Phe^{6}Gln^{34}hPP, pNPY(1 9-23)-Phe^{6}His^{34}hPP, pNPY(I -7, 19-23)-hPP, pNPY(l -7, 19-23)-Gln^{34}hPP, cPP(20-23)-Pr$_{O}$^{34}-pNPY, cPP(21-23)-Pr$_{O}$^{34}-pNPY, cPP(22-23)-Pro^{34}-pNPY, cPP(l-7)-Pro^{34}-pNPY, cPP(20-23)-Pro^{34}-pNPY, cPP(l-7,20-23)-Pro ^{34}-pNPY, cPP(l-7)-pNPY(19-23)-hPP, cPP(l-7)-pNPY(19-23)-His ^{34}hPP, or cPP(l-7)-gPP(19-23)-hPP.

In another embodiment, such PPF polypeptides of the invention also do not include: Thr^{27}hPYY(3-36), Ile^{30}hPYY(3-36), Ser^{32}hPYY(3-36), Lys^{33}hPYY(3-36), Asn^{34}hPYY(3-36), Lys^{3s}hPYY(3-36), Thr^{36}hPYY(3-36), Lys^{25}Thr^{27}hPYY(3-36), Lys^{25}Ile^{30}hPYY(3-36), Lys^{25}Ser^{32}hPYY(3-36), Lys^{25}Lys^{33}hPYY(3-36), Lys^{25}Asn^{24}hPYY(3-36), Lys^{25}Lys^{35}hPYY(3-36), Lys^{25}Thr^{36}hPYY(3-36), Thr^{27}Ile^{28}hPYY(3-36), Thr^{27}Val^{28}hPYY(3-36), Thr^{27}Gln^{29}hPYY(3-36), Thr^{27}Ile^{30}hPYY(3-36), Thr^{27}Val^{30}hPYY(3-36), Thr^{27}Ile^{31}hPYY(3-36), Thr^{27}Leu^{31}hPYY(3-36), Thr^{27}Ser^{32}hPYY(3-36), Thr^{27}Lys^{33}hPYY(3-36),

Thr^{27}Asn^{34}hPYY(3-36), Thr^{27}Lys^{35}hPYY(3-36), Thr^{27}Thr^{36}hPYY(3-36), Thr^{27}Phe^{36}hPYY(3-36), Phe^{27}Ile^{30}hPYY(3-36), Phe^{27}Ser^{32}hPYY(3-36), Phe^{27}Lys^{33}hPYY(3-36), Phe^{27}Asn^{34}hPYY(3-36), Phe^{27}Lys^{35}hPYY(3-36), Phe^{27}Thr^{36}hPYY(3-36), Gln^{29}Ile^{30}hPYY(3-36), Gln^{29}Ser^{32}hPYY(3-36), Gln^{29}Leu^{33}hPYY(3-36), Gln^{29}Asn^{34}hPYY(3-36), Gln^{29}Leu^{35}hPYY(3-36), Gln^{29}Thr^{36}hPYY(3-36), He^{30}IIe^{31}IiPYY(S-SO), Ile^{30}Leu^{31}hPYY(3-36), Ile^{30}Ser^{32}hPYY(3-36), Ile^{30}Lys^{33}hPYY(3-36), Ile^{30}Asn^{34}hPYY(3-36), Ile^{30}Lys^{35}hPYY(3-36), Ile^{30}Thr^{36}hPYY(3-36), Ile^{30}Phe^{36}hPYY(3-36), Val^{30}Ser^{32}hPYY(3-36), Val^{30}Lys^{33}hPYY(3-36), Val^{30}Asn^{34}hPYY(3-36), Val^{30}Lys^{35}hPYY(3-36), Val^{30}Thr^{36}hPYY(3-36), He^{31}SCr^{32}IiPYY(S-SO), Ile^{31}Lys^{33}hPYY(3-36), Ile^{31}Asn^{34}hPYY(3-36), Ile^{31}Lys^{35}hPYY(3-36), Ile^{31}Thr^{36}hPYY(3-36), He^{31}PIle^{36}IiPYY(S-So)$_5$ Leu^{31}Ser^{32}hPYY(3-36), Leu^{31}Lys^{33}hPYY(3-36), Leu^{31}Asn^{34}hPYY(3-36), Leu^{31}Lys^{35}hPYY(3-36), Leu^{31}Thr^{36}hPYY(3-36), Ser^{32}Lys^{33}hPYY(3-36), Ser^{32}Asn^{34}hPYY(3-36), Ser^{32}Lys^{35}hPYY(3-36), Ser^{32}Thr^{36}hPYY(3-36), Ser^{32}Phe^{36}hPYY(3-36), Lys^{33}Asn^{34}hPYY(3-36), Lys^{33}Lys^{35}hPYY(3-36), Lys^{33}Thr^{36}hPYY(3-36), Lys^{33}Phe^{36}hPYY(3-36), Asn^{34}Lys^{35}hPYY(3-36), Asn^{34}Phe^{36}hPYY(3-36), Lys^{35}Thr^{36}hPYY(3-36), Lys^{35}Phe^{36}hPYY(3-36), Thr^{27}hPYY(4-36), Phe^{27}hPYY(4-36), Ile^{28}hPYY(4-36), Val^{28}hPYY(4-36), Gln^{29}hPYY(4-36), Ile^{30}hPYY(4-36), Val^{30}hPYY(4-36), Ile^{31}hPYY(4-36), Leu^{31}IiPYY(4-36), Ser^{32}hPYY(4-36), Lys^{33}hPYY(4-36), Asn^{34}hPYY(4-36), Lys^{35}hPYY(4-36), Thr^{36}hPYY(4-36), Phe^{36}hPYY(4-36), Lys^{25}Thr^{27}hPYY(4-36), Lys^{25}Phe^{27}hPYY(4-36), Lys^{25}Ile^{28}hPYY(4-36), Lys^{25}Val^{2s}hPYY(4-36), Lys^{25}Gln^{29}hPYY(4-36), LyS^{25}IIe^{30}IiPYY(4-36), Lys^{25}Val^{30}hPYY(4-36), Lys^{25}Ile^{31}hPYY(4-36), Lys^{25}Leu^{31}hPYY(4-36), Lys^{25}Ser^{32}hPYY(4-36), Lys^{25}Lys^{33}hPYY(4-36), Lys^{25}Asn^{24}hPYY(4-36), Lys^{25}Lys^{35}hPYY(4-36), Lys^{25}Thr^{36}hPYY(4-36), Lys^{25}Phe^{36}hPYY(4-36), Thr^{27}Ile^{28}hPYY(4-36), Thr^{27}Val^{28}hPYY(4-36), Thr^{27}Gln^{29}hPYY(4-36), ThT^{27}IIe^{30}IiPYY(4-36), Thr^{27}Val^{30}hPYY(4-36), Thr^{27}Ile^{31}hPYY(4-36), Thr^{27}Leu^{31}hPYY(4-36), Thr^{27}Ser^{32}hPYY(4-36), Thr^{27}Lys^{33}hPYY(4-36), Thr^{27}Asn^{34}IiPYY(4-36), Thr^{27}Lys^{35}hPYY(4-36), Thr^{27}Thr^{36}hPYY(4-36), Thr^{27}Phe^{36}hPYY(4-36), Phe^{27}Ile^{28}hPYY(4-36), Phe^{27}Val^{28}hPYY(4-36), Phe^{27}Gln^{29}hPYY(4-36), Phe^{27}Ile^{30}hPYY(4-36), Phe^{27}Val^{30}hPYY(4-36), Phe^{27}Ile^{31}hPYY(4-36), Phe^{27}Leu^{31}hPYY(4-36),

Phe^{27}Ser^{32}hPYY(4-36), Phe^{27}Lys^{33}hPYY(4-36), Phe^{27}Asn^{34}hPYY(4-36), Phe^{27}Lys^{35}hPYY(4-36), Phe^{27}Thr^{36}liPYY(4-36), Phe^{27}Phe^{36}hPYY(4-36), Gln^{29}Ile^{30}hPYY(4-36), Gln^{29}Val^{30}hPYY(4-36), GIn^{29}IIe^{31}IiPYY(4-36), GIn^{29}LeU^{31}IiPYY(4-36), Gln^{29}Ser^{32},hPYY(4-36) Gln^{29}Leu^{33}hPYY(4-36), Gln^{29}Asn^{34}hPYY(4-36), Gln^{29}Leu^{35}hPYY(4-36), Gln^{29}Thr^{36}hPYY(4-36), Gln^{29}Phe^{36}hPYY(4-36), He^{30}IIe^{31}IiPYY(4-36), Ile^{30}Leu^{31}hPYY(4-36), Ile^{30}Ser^{32}hPYY(4-36), Ile^{30}Lys^{33}hPYY(4-36), Ile^{30}Asn^{34}hPYY(4-36), Ile^{30}Lys^{35}hPYY(4-36), Ile^{30}Thr^{36}hPYY(4-36), Ile^{30}Phe^{36}hPYY(4-36), Val^{30}Ile^{31}hPYY(4-36), VaI^{30}LeU^{31}IiPYY(4-36), Val^{30}Ser^{32}hPYY(4-36), Val^{30}Lys^{33}hPYY(4-36), Val^{30}Asn^{34}hPYY(4-36), Val^{30}Lys^{35}hPYY(4-36), Val^{30}Tlir^{36}hPYY(4-36), Val^{30}Phe^{36}hPYY(4-36), Ile^{31}Ser^{32}hPYY(4-36), He^{31}LyS^{33}IiPYY(4-36), He^{31}ASn^{34}IiPYY(4-36), He^{31}LyS^{35}IiPYY(4-36), He^{31}ThT^{36}IiPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Ser^{32}hPYY(4-36), VaI^{31}LyS^{33}IiPYY(4-36), Leu^{31}Asn^{34}hPYY(4-36), Leu^{31}Lys^{35}hPYY(4-36), Leu^{31}Thr^{36}hPYY(4-36), Leu^{31}Phe^{36}IiPYY(4-36), Ser^{32}Lys^{33}hPYY(4-36), Ser^{32}Asn^{34}hPYY(4-36), Ser^{32}Lys^{35}hPYY(4-36), Ser^{32}Thr^{36}hPYY(4-36), Ser^{32}Phe^{36}hPYY(4-36), Lys^{33}Asn^{34}liPYY(4-36), Lys^{33}Lys^{35}hPYY(4-36), Lys^{33}Thr^{36}hPYY(4-36), Lys^{33}Phe^{36}hPYY(4-36), Asn^{34}Lys^{35}hPYY(4-36), Asn^{34}Phe^{36}hPYY(4-36), Lys^{35}Thr^{36}hPYY(4-36), Lys^{35}Phe^{36}hPYY(4-36), Thr^{27}hPYY(5-36), Phe^{27}hPYY(5-36), Ile^{28}hPYY(5-36), Val^{28}hPYY(5-36), Gln^{29}hPYY(5-36), Ile^{30}hPYY(5-36), Val^{30}hPYY(5-36), Ile^{31}hPYY(5-36), Leu^{31}IiPYY(S-SO), Ser^{32}hPYY(5-36), Lys^{33}hPYY(5-36), Asn^{34}hPYY(5-36), Lys^{35}hPYY(5-36), Thr^{36}hPYY(5-36), Phe^{36}hPYY(5-36), Lys^{25}Thr^{27}hPYY(5-36), Lys^{25}Phe^{27}liPYY(5-36), Lys^{25}Ile^{28}hPYY(5-36), Lys^{25}Val^{28}hPYY(5-36), Lys^{25}Gln^{29}hPYY(5-36), Lys^{25}Ile^{30}hPYY(5-36), Lys^{25}Val^{30}hPYY(5-36), LyS^{25}IIe^{31}IiPYY(S-SO), Lys^{25}Leu^{31}hPYY(S-3O), Lys^{25}Ser^{32}hPYY(5-36), Lys^{25}Lys^{33}hPYY(5-36), Lys^{25}Asn^{24}hPYY(5-36), Lys^{25}Lys^{35}hPYY(5-36), Lys^{25}Thr^{36}hPYY(5-36) , Lys^{25}Phe^{36}hPYY(5-36), Thr^{27}Ile^{28}hPYY(5-36), Thr^{27}Val^{28}hPYY(5-36), Thr^{27}Gln^{29}hPYY(5-36), Thr^{27}Ile^{30}hPYY(5-36), Thr^{27}Val^{30}hPYY(5-36), Thr^{27}Ile^{31}hPYY(5-36), Thr^{27}Leu^{31}hPYY(5-36), Thr^{27}Ser^{32}hPYY(5-36), Thr^{27}Lys^{33}hPYY(5-36), Thr^{27}Asn^{34}hPYY(5-36), Thr^{27}Lys^{35}hPYY(5-36), Thr^{27}Thr^{36}hPYY(5-36), Thr^{27}Phe^{36}hPYY(5-36), Phe^{27}Ile^{28}hPYY(5-36), Phe^{27}Val^{28}hPYY(5-36),

Phe^{27}Gln^{29}hPYY(5-36), Phe^{27}Ile^{30}hPYY(5-36), Phe^{27}Val^{30}hPYY(5-36), Phe^{27}Ile^{31}hPYY(5-36), Phe^{27}Leu^{31}hPYY(5-36), Phe^{27}Ser^{32}hPYY(5-36), Phe^{27}Lys^{33}hPYY(5-36), Phe^{27}Asn^{34}hPYY(5-36), Phe^{27}Lys^{35}hPYY(5-36), Phe^{27}Thr^{36}hPYY(5-36), Phe^{27}Phe^{36}hPYY(5-36), Gln^{29}Ile^{30}hPYY(5-36), Gln^{29}Val^{30}hPYY(5-36), GIn^{29}IIe^{31}IiPYY(S-SO), Gln^{29}Leu^{31}hPYY(5-36), Gln^{29}Ser^{32},hPYY(5-36) Gln^{29}Leu^{33}hPYY(5-36), Gln^{29}Asn^{34}hPYY(5-36), Gln^{29}Leu^{35}hPYY(5-36), GIn^{29}ThT^{36}IiPYY(5-36), Gln^{29}Phe^{36}hPYY(5-36), He^{30}IIe^{31}IiPYY(S-So), Ile^{30}Leu^{31}hPYY(5-36), Ile^{30}Ser^{32}hPYY(5-36), Ile^{30}Lys^{33}hPYY(5-36), He^{30}Asn^{34}liPYY(5-36), Ile^{30}Lys^{35}hPYY(5-36), Ile^{30}Thr^{36}hPYY(5-36), Ile^{30}Phe^{36}hPYY(5-36), Val^{30}Ile^{31}hPYY(5-36), VaI^{30}LeU^{31}IiPYY(S-So), Val^{30}Ser^{32}hPYY(5-36), Val^{30}Lys^{33}hPYY(5-36), Val^{30}Asn^{34}hPYY(5-36), Val^{30}Lys^{35}hPYY(5-36), Val^{30}Thr^{36}hPYY(5-36), Val^{30}Phe^{36}hPYY(5-36), He^{31}SeT^{32}IiPYY(S-So), Ile^{31}Lys^{33}hPYY(5-36), He^{31}ASn^{3}VYY(S-SO), Ile^{31}Lys^{35}hPYY(5-36), Ile^{31}Thr^{36}nPYY(5-36), Leu^{31}Phe^{36}hPYY(5-36), Leu^{31}PhC^{3}VYY(S-SO), Leu^{31}Ser^{32}hPYY(5-36), Val^{31}Lys^{33}hPYY(5-36), Leu^{31}ASn^{3}VYY(S-So), Leu^{31}LyS^{3}VYY(S-So), Leu^{31}ThT^{36}IiPYY(S-SO), Leu^{31}PhC^{3}VYY(S-So), Ser^{32}Lys^{33}hPYY(5-36), Ser^{32}Asn^{34}hPYY(5-36), Ser^{32}Lys^{35}hPYY(5-36), Ser^{32}Thr^{36}hPYY(5-36), Ser^{32}Phe^{36}hPYY(5-36), Lys^{33}Asn^{34}hPYY(5-36), Lys^{33}Lys^{35}hPYY(5-36), Lys^{33}Thr^{36}hPYY(5-36), Lys^{33}Phe^{36}hPYY(5-36), Asn^{34}Lys^{35}hPYY(5-36), Asn^{34}Phe^{36}hPYY(5-36), Lys^{35}Thr^{36}hPYY(5-36), or Lys^{35}Phe^{36}hPYY(5-36).

In another embodiment, the PPF polypeptides of the invention do not include any unnatural amino acid residues, and preferably comprise a C-terminal tail motif of hPYY. The C-terminal tail motif may preferably comprise amino acid residues 32-35 of hPYY, *e.g.,* Thr, Arg, GIn, Arg (SEQ ID NO: 351). In such an embodiment, the PPF polypeptides of the invention do not include any native PPF polypeptides *(e.g.,* NPY(l-36), NPY(3-36), PYY(l-36), PYY(3-36)), NPY(2-36), PYY(4-36), PYY(5-36)), (2-36)NPY, (2-36)PYY, GIn^{3}VP, He^{31}GIn^{34}PP, AIa^{1}NPY, Tyr^{1}NPY, AIa^{2}NPY, LeU^{2}NPY, Phe^{2}NPY, HiS^{2}NPY, AIa^{3}NPY, AIa^{4}NPY, AIa^{6}NPY, Tyr^{7}pNPY, AIa^{7}NPY, AIa^{9}NPY, AIa^{10}NPY, AIa^{11}NPY, GIy^{12}NPY, AIa^{13}NPY, GIy^{14}NPY, AIa^{15}NPY, AIa^{16}NPY, AIa^{17}NPY, GIy^{18}NPY AIa^{19}NPY, LyS^{19}NPY, AIa^{20}NPY$_{5}$ AIa^{21}NPY, AIa^{22}NPY, GIy^{23}NPY, AIa^{24}NPY, Trp^{24}pNPY, AIa^{25}NPY, LyS^{25}NPY, AIa^{26}NPY,

AIa^{27}NPY, Phe^{27}NPY, AIa^{28}NPY, AIa^{29}NPY, GIn^{29}NPY, AIa^{30}NPY, Phe^{30}NPY, AIa^{31}NPY, Trp^{31}pNPY, AIa^{36}NPY, Phe^{36}NPY, HiS^{36}NPY, Ahx(9-22)pNPY, Ahx(9-17)pNPY, des-AA(10-17)-pNPY, des-AA(10-17)-Cys$^{2'27}$-pNPY des-AA(10-17)-Ala$^{7'21}$-pNPY, des-AA(10-17)-Cys$^{7'21}$-pNPY, des-AA(10-17)-Glu^{7}Lys^{2}^pNPY, Lys^{25}hPYY(5-36), Arg^{4}hPYY(4-36), Gln^{4}hPYY(4-36), Asn^{4}hPYY(4-36), Lys^{25}hPYY(4-36), Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Tyr^{U6}pPYY, Pro^{13}Ala^{14}hPYY, FMS-PYY, FMS-PYY(3-36), Fmoc-PYY, Fmoc-PYY(3-36), FMS$_2$-PYY, FMS$_2$-PYY(3-36), Fmoc$_2$-PYY, Fmoc$_2$-PYY(3-36), hPP(l-7)-pNPY, liPP(l-17)-pNPY, hPP(19-23)-pNPY, rPP(19-23)-pNPY, hPP(l-7)-pNPY, hPP(l-17)-pNPY, hPP(19-23)-pNPY, rPP(19-23)-pNPY, pNPY(19-23)-Gln^{34}hPP, pNPY(19-23)-Phe^{6}Gln^{34}hPP, or pNPY(l-7,19-23)-Gln ^{34}hPP.

In another aspect, such PPF polypeptides of the invention comprising a C-terminal tail motif of hPYY also do not include: Thr^{27}hPYY(3-36), Ile^{30}hPYY(3-36), Thr^{36}hPYY(3-36), Lys^{25}Thr^{27}hPYY(3-36), Lys^{25}Ile^{30}hPYY(3-36), Lys^{25}Asn^{24}hPYY(3-36), Lys^{25}Thr^{36}hPYY(3-36), Thr^{27}Ile^{28}hPYY(3-36), Thr^{27}Val^{28}hPYY(3-36), Thr^{27}Gln^{29}hPYY(3-36), Thr^{27}Ile^{30}hPYY(3-36), Thr^{27}Val^{30}hPYY(3-36), Thr^{27}Ile^{31}hPYY(3-36), Thr^{27}Leu^{31}hPYY(3-36), Thr^{27}Thr^{36}hPYY(3-36), Thr^{27}Phe^{36}hPYY(3-36), Phe^{27}Ile^{30}hPYY(3-36), Phe^{27}Thr^{36}hPYY(3-36), Gln^{29}Ile^{30}hPYY(3-36), Gln^{29}Thr^{36}hPYY(3-36), He^{30}IIe^{31}IiPYY(S-SO), He^{30}LeU^{31}IiPYY(S-SO), Ile^{30}Thr^{36}hPYY(3-36), Ile^{30}Phe^{36}hPYY(3-36), Val^{30}Thr^{36}hPYY(3-36), He^{31}ThT^{36}IiPYY(S-SO), Ile^{31}Phe^{36}hPYY(3-36), Leu^{31}Thr^{36}hPYY(3-36), Thr^{27}hPYY(4-36), Phe^{27}hPYY(4-36), Ile^{28}hPYY(4-36), VaI^{28}IiPYY(4-36), GIn^{29}IiPYY(4-36), Ile^{30}hPYY(4-36), Val^{30}hPYY(4-36), Ile^{31}hPYY(4-36), Leu^{31}hPYY(4-36), Thr^{36}hPYY(4-36), Phe^{36}hPYY(4-36), Lys^{25}Thr^{27}hPYY(4-36), Lys^{25}Phe^{27}hPYY(4-36), Lys^{25}Ile^{28}hPYY(4-36), Lys^{25}Val^{28}hPYY(4-36), Lys^{25}Gln^{29}hPYY(4-36), Lys^{25}Ile^{30}hPYY(4-36), Lys^{25}Val^{30}hPYY(4-36), Lys^{25}Ile^{31}hPYY(4-36), Lys^{25}Leu^{31}IiPYY(4-36), Lys^{25}Thr^{36}liPYY(4-36), Lys^{25}Phe^{36}hPYY(4-36), Thr^{27}Ile^{28}hPYY(4-36), Thr^{27}Val^{28}hPYY(4-36), Thr^{27}Gln^{29}hPYY(4-36), Thr^{27}Ile^{30}hPYY(4-36), Thr^{27}Val^{30}hPYY(4-36), Thr^{27}Ile^{31}hPYY(4-36), Thr^{27}Leu^{31}hPYY(4-36), Thr^{27}Thr^{36}hPYY(4-36), Thr^{27}Phe^{36}hPYY(4-36), Phe^{27}Ile^{28}hPYY(4-36), Phe^{27}Val^{28}hPYY(4-36), Phe^{27}Gln^{29}hPYY(4-36),

Phe^{27}Ile^{30}hPYY(4-36), Phe^{27}Val^{30}hPYY(4-36), Phe^{27}Ile^{31}hPYY(4-36), Phe^{27}Leu^{31}hPYY(4-36), Phe^{27}Thr^{36}hPYY(4-36), Phe^{27}Phe^{36}hPYY(4-36), GIn^{29}IIe^{30}IiPYY(4-36), Gln^{29}Val^{30}hPYY(4-36), GIn^{29}IIe^{31}IiPYY(4-36), GIn^{29}LeU^{31}IiPYY(4-36), Gln^{29}Thr^{36}hPYY(4-36), Gln^{29}Phe^{36}hPYY(4-36), He^{30}IIe^{31}IiPYY(4-36), Ue^{30}LeU^{31}IiPYY(4-36), Ile^{30}Thr^{36}hPYY(4-36), Ile^{30}Phe^{36}hPYY(4-36), VaI^{30}IIe^{31}IiPYY(4-36), VaI^{30}LeU^{31}IiPYY(4-36), Val^{30}Thr^{36}hPYY(4-36), Val^{30}Phe^{36}hPYY(4-36), Ile^{31}Thr^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Thr^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Thr^{27}hPYY(5-36), Phe^{27}hPYY(5-36), Ile^{28}liPYY(5-36), Val^{28}hPYY(5-36), Ghr^{29}hPYY(5-36), Ile^{30}hPYY(5-36), Val^{30}hPYY(5-36), lie^{31}IiPYY(S-So), Leu^{31}hPYY(5-36), Thr^{36}hPYY(5-36), Phe^{36}hPYY(5-36), Lys^{25}Thr^{27}hPYY(5-36), Lys^{25}Phe^{27}hPYY(5-36), LyS^{25}IIe^{28}IiPYY(5-36), Lys^{25}Val^{28}hPYY(5-36), Lys^{25}Gln^{29}hPYY(5-36), LyS^{25}IIe^{30}IiPYY(5-36), LyS^{25}VaI^{30}IiPYY(5-36), Lys^{25}Ile^{31}hPYY(5-36), Lys^{25}Leu^{31}hPYY(5-36), Lys^{25}Thr^{36}liPYY(5-36), Lys^{25}Phe^{36}hPYY(5-36), Thr^{27}Ile^{28}hPYY(5-36), Thr^{27}Val^{28}hPYY(5-36), Thr^{27}Gln^{29}hPYY(5-36), Thr^{27}Ile^{30}hPYY(5-36), Thr^{27}Val^{30}hPYY(5-36), ThT^{27}IIe^{31}IiPYY(S-So), Thr^{27}Leu^{31}IiPYY(S-SO), Thr^{27}Thr^{36}hPYY(5-36), Thr^{27}Phe^{36}hPYY(5-36), Phe^{27}Ile^{28}hPYY(5-36), Phe^{27}Val^{28}hPYY(5-36), Phe^{27}Gln^{29}liPYY(5-36), Phe^{27}Ile^{30}hPYY(5-36), Phe^{27}Val^{30}hPYY(5-36), Phe^{27}lle^{31}IiPYY(S-SO), Phe^{27}Leu^{31}IiPYY(S-SO), Phe^{27}Thr^{36}hPYY(5-36), Phe^{27}Phe^{36}hPYY(5-36), GIn^{29}IIe^{30}IiPYY(5-36), GIn^{29}VaI^{3}V YY(5-36), GIn^{29}IIe^{31}IiPYY(S-So), Gln^{29}Leu^{31}IiPYY(S-SO), Gln^{29}Thr^{36}hPYY(5-36), Gln^{29}Phe^{36}liPYY(5-36), He^{30}IIe^{31}IiPYY(S-SO), Ile^{30}Leu^{31}hPYY(5-36), He^{30}ThT^{36}IiPYY(5-36), Ile^{30}Phe^{36}liPYY(5-36), VaI^{30}IIe^{31}IiPYY(S-So), Val^{30}Leu^{31}hPYY(5-36), Val^{30}Thr^{36}hPYY(5-36), Val^{30}Phe^{36}liPYY(5-36), Ile^{31}Thr^{36}hPYY(5-36), Leu^{31}Phe^{3}VYY(S-SO), Leu^{31}PhC^{36}IiPYY(S-SO), Leu^{31}ThT^{36}IiPYY(S-SO)$_5$ Leu^{31}Phe^{36}IiPYY(S-So).

In yet another emobodiment, the PPF polypeptides of the invention do not include those PPF-related polypeptides disclosed in WO 03/026591 and WO 03/057235, which are herein incorporated by reference in their entirety.

In another embodiment, the polypeptides of the invention are at least 34 amino acids in length. In other embodiments, the PPF polypeptides may be at least 21, 22, 23, 24,

25, 26, 27, 28, 29, 30, 31, 32, or 33 amino acids in length. Further, in one embodiment, the polypeptides of the invention include only natural L amino acid residues and/or modified natural L amino acid residues. Alternatively, in another embodiment, the polypeptides of the invention do not include unnatural amino acid residues.

In yet another embodiment, PPF polypeptides of the invention may exhibit at least 60%, 65%, 70%, 80%, or 90% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). Such PPF polypeptides of the invention may also exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native PP. In yet another embodiment, such PPF polypeptides of the invention may exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native NPY.

More specifically, in a first aspect, the present invention relates to novel PPF polypeptides comprising at least two PPF motifs, wherein the at least two PPF motifs include at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif, and the PPF polypeptide does not include any unnatural amino acid residues. Such PPF polypeptides of the invention will exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). In a preferred embodiment, such PPF polypeptides have at least 34 amino acid residues. In another preferred embodiment, such PPF polypeptides of the invention may exhibit at least 60%, 65%, 70%, 80%, or 90% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). Such PPF polypeptides of the invention may also exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native PP. In yet another embodiment, such PPF polypeptides of the invention may exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native NPY.

In another aspect, the PPF polypeptides of the invention include PYY analog polypeptides. In yet another aspect of the invention, the PPF polypeptides of the invention include PPF chimeric polypeptides comprising a fragment of a PP, PYY or NPY polypeptide covalently linked to at least one additional fragment of a PP, PYY or NPY polypeptide, wherein each PP, PYY or NPY fragment includes a PPF motif. Such PPF analog polypeptides and PPF chimeric polypeptides of the invention will exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). In a preferred embodiment, such PPF polypeptides of the invention

may exhibit at least 60%, 65%, 70%, 80%, or 90% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). PPF polypeptides of the invention may also exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native PP. In yet another embodiment, PPF polypeptides of the invention may exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native NPY. In certain embodiments, desirable PPF polypeptides may not include N-terminal PP fragments in combination with C-terminal NPY fragments.

By way of background, PYY, NPY, and PP constitute a family of C-terminally amidated peptides involved in the regulation of gastrointestinal function, blood pressure, and feeding behavior. Without intending to be limited by theory, the ability of these peptides to selectively bind and activate Y receptor subtypes is believed to depend strongly on a stable solution structure, including the so-called "PP-fold". Table 1 (below) shows PP family ligand potencies at the known receptors and the rank order of potencies of various ligands.

TABLE 1. Summary of receptor pharmacology for the PP family of receptors

RECEPTORS	PHARMACOLOGY	REFERENCE
Food Intake Inhibition (peripheral)	PYY(3-36) ≥ PYY >> NPY , NPY(3-36) , PP , Ac-PYY(22-36)	
Gastric Emptying	PYY(3-36) ≥ PYY >> NPY , NPY(3-36) , PP , Ac-PYY(22-36)	
Food Intake Stimulation (central)	PYY ≥ PYY(3-36) = NPY = NPY(3-36) > PP	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999
Y1	NPY = PYY > NPY(3-36) = PYY(3-36) = PP	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999; Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998; US 5,968,819
Y2	NPY = PYY = PYY(3-36) = NPY(3-36) >> PP	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999; Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998; US 5,968,819
Y3	NPY > PP > PYY	Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998.
Y4	PP > PYY > NPY > PYY(3-36) = NPY(3-36)	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999; Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998; US 5,968,819
Y5	NPY = PYY ≥ PP ≥ PYY(3-36) = NPY(3-36)	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999; Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998; US 5,968,819
Y6	NPY = PYY ≥ NPY(3-36) > PP	Iyengar et al., J. Pharmacol. Exp. Ther. 289: 1031-40, 1999; Gehlert, Proc. Soc. Exp. Biol. Med. 218: 7-22, 1998; Michel et al., Pharmacol. Rev. 50: 143-50, 1998; US 5,968,819
(Y7)	PYY > NPY >> PYY(3-36) = PP	Yang et al., Br. J. Pharmacol. 123: 1549-54, 1998
(Y7)	PYY(3-36) ≥ PYY > NPY >> PP	Haynes et al., Br. J. Pharmacol. 122: 1530-6, 1997
(Y7)	PYY >> NPY = PYY(3-36) = PP	Kawakubo et al., Brain Res. 854: 30-4, 2000

Research has suggested that the differences in Y receptor binding affinities are correlated with secondary and tertiary structural differences. *See, e.g.,* Keire *et al, Biochemistry* 2000, 39, 9935-9942. Native porcine PYY has been characterized as including two C-terminal helical segments from residues 17 to 22 and 25 to 33 separated by a kink at residues 23, 24, and 25, a turn centered around residues 12-14, and the N-terminus folded near residues 30 and 31. Further, full-length porcine PYY

has been characterized as including the PP fold, stabilized by hydrophobic interactions among residues in the N- and C- termini. *See id.*

By "PP" is meant a pancreatic peptide polypeptide obtained or derived from any species. Thus, the term "PP" includes both the human full length, 36 amino acid peptide as set forth in SEQ ID NO: 1, and species variations of PP, including, *e.g.*, murine, hamster, chicken, bovine, rat, and dog PP. In this sense, "PP," "wild-type PP," and "native PP," *i.e.*, unmodified PP, are used interchangeably.

By "NPY" is meant a neuropeptide Y polypeptide obtained or derived from any species. Thus, the term "NPY" includes both the human full length, 36 amino acid peptide as set forth in SEQ ID NO: 4, and species variations of NPY, including, *e.g.*, murine, hamster, chicken, bovine, rat, and dog NPY. In this sense, "NPY," "wild-type NPY," and "native NPY", *i.e.*, unmodified NPY, are used interchangeably.

By "PYY" is meant a peptide YY polypeptide obtained or derived from any species. Thus, the term "PYY" includes both the human full length, 36 amino acid peptide as set forth in SEQ ID NO: 2, and species variations of PYY, including *e.g.*, murine, hamster, chicken, bovine, rat, and dog PYY. In this sense, "PYY" and "wild-type PYY" and "native PYY," *i.e.*, unmodified PYY, are used interchangeably. In the context of the present invention, all modifications discussed with reference to the PYY analog polypeptides of the present invention are based on the 36 amino acid sequence of native human PYY (SEQ ID NO: 2).

By "PP agonist", "PYY agonist", or "NPY agonist" is meant a compound which elicits a biological activity of native human PP, PYY, or NPY, respectively. In a preferred embodiment, the terms refer to a compound which elicits a biological effect in the reduction of nutrient availability similar to that of native human PP, PYY, or NPY, for example a compound (1) having activity in the food intake, gastric emptying, pancreatic secretion, or weight loss assays similar to native human PP, PYY, or NPY, and (2) which binds specifically in a Y receptor assay or in a competitive binding assay with labeled PP, PYY, PYY(3-36), or NPY from certain tissues having an abundance of Y receptors, including, *e.g.*, area postrema. In a preferred embodiment, the agonist is not PP, PYY, PYY(3-36), and/or NPY. Preferably, the agonists will bind in such assays with an affinity of greater than 1 μM,

and more preferably with an affinity of greater than 1-5 nM. Such agonists may comprise a polypeptide having a PPF motif, an active fragment of PP, PYY, or NPY, or a small chemical molecule.

By "amino acid" and "amino acid residue" is meant natural amino acids, unnatural amino acids, and modified amino acid. Unless stated to the contrary, any reference to an amino acid, generally or specifically by name, includes reference to both the D and the L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (GIn), glutamic acid (GIu), glycine (GIy), histidine (His), isoleucine (He), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (VaI). Unnatural amino acids include, but are not limited to homolysine, homoarginine, azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine, 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylalanine, N-methylglycine, N-methylisoleucine, N-methylpentylglycine, N-methylvaline, naphthalanine, norvaline, norleucine, ornithine, pentylglycine, pipecolic acid, thioproline, sarcosine and citrulline. Additional unnatural amino acids include modified amino acid residues which are chemically blocked, reversibly or irreversibly, or chemically modified on their N-terminal amino group or their side chain groups, as for example, N-methylated D and L amino acids or residues wherein the side chain functional groups are chemically modified to another functional group. For example, modified amino acids include methionine sulfoxide; methionine sulfone; aspartic acid- (beta-methyl ester), a modified amino acid of aspartic acid; N-ethylglycine, a modified amino acid of glycine; or alanine carboxamide, a modified amino acid of alanine. Additional residues that can be incorporated are described in Sandberg *et ah, J. Med. Chem.* 41: 2481-91, 1998.

By "Ahx" is meant 6-amino hexanoic acid.

Certain human sequences of peptides in the PPF are as follows (in conventional one-letter amino acid code):

PP: APLEPVYPGD NATPEQMAQY AADLRRYINM LTRPRY **(SEQ ID NO: 1)**
PYY: YPIKPEAPGE DASPEELNRY YASLRHYLNL VTRQRY **(SEQIDNO: 2)**
PYY(3-36): IKPEAPGE DASPEELNRY YASLRHYLNL VTRQRY **(SEQIDNO: 3)**
NPY: YPSKPDNPGE DAPAEDMARY YSALRHYINL ITRQRY **(SEQIDNO: 4)**

Species homologs of human PYY include those amino acid sequences of SEQ ID NOs. 7-29.

As mentioned above, these peptides are C-terminally amidated when expressed physiologically, but need not be for the purposes of the instant invention. In other words, the C-terminus of these peptides, as well as the PPF polypeptides of the present invention, may have a free -OH or $-NH_2$ group. These peptides may also have other post-translational modifications. One skilled in the art will appreciate that the PPF polypeptides of the present invention may also be constructed with an N-terminal methionine residue.

Preferred PPF polypeptides of the invention include the PPF polypeptides of the Formula (I) (SEQ ID NO: 30):

X aa*i* Xaa_2 Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaa_9 Xaa_{10}
Xaaπ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Xaa_{25} Xaa_{26} Xaa_{27} Xaa_{28} Xaa_{29} Xaa_{30}
Xaa_{31} Thr Arg GIn Arg Xaa_{36}

wherein:
Xaai is Tyr, Ala, Phe, Trp, or absent;
Xaa_2 is Pro, GIy, d-AIa, homoPro, hydroxyPro, or absent;
Xaa_3 is He, Ala, NorVal, VaI, Leu, Pro, Ser, Thr or absent;
Xaa_4 is Lys, Ala, GIy, Arg, d-Ala, homoLys, homo-Arg, GIu, Asp, or absent;
Xaa_6 is GIu, Ala, VaI, Asp, Asn, or GIn;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaag is GIy, Ala Ser, sarcosine, Pro, or Aib;
Xaa_{10} is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaaπ is Asp, Ala, GIu, Asn, GIn, Pro, Aib, or GIy;
$Xaai_2$ is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, Pro, or homoSer;
Xaa_{14} is Pro, Ala, homo-Pro, hydroxyPro, Aib, or GIy;
Xaa_{15} is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaa_{16} is GIu, Ala, Asp, Asn, or GIn;
Xaa_{17} is Leu, Ala, Met, Trp, He, VaI, or NorVal;

Xaa$_{18}$ is Asn, Asp, Ala, GIu, GIn, Ser, or Thr;
Xaa$_{19}$ is Arg, Tyr, Lys, Ala, GIn, or N(Me)AIa;
Xaa$_{2}$i is Tyr, Ala, Met, Phe, or Leu;
Xaa$_{22}$ is Ala, Ser, Thr, or d-Ala;
Xaa$_{23}$ is Ser, Ala, Asp, Thr, or homoSer;
Xaa$_{25}$ is Arg, homoArg, Lys, homoLys, Orn, or Cit;
Xaa$_{26}$ is His, Ala, Arg, homoArg, homoLys, Orn, or Cit;
Xaa$_{27}$ is Tyr or Phe;
Xaa$_{28}$ is Leu, He, VaI, or Ala;
Xaa$_{29}$ is Asn or GIn;
Xaa$_{30}$ is Leu, Ala, NorVal, VaI, He, or Met;
Xaa$_{31}$ is Ala, VaI, lie, or Leu; and
Xaa$_{36}$ is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said PPF polypeptide is not a native PPF polypeptide, NPY(2-36), NPY(4-36), PYY(2-36), PYY(4-36), PP(2-36), PP(4-36), AIa^{1}NPY, AIa^{3}NPY, AIa^{4}NPY, AIa^{6}NPY, AIa^{7}NPY, Tyr^{7}pNPY, AIa^{9}NPY, AIa^{10}NPY, AIa^{11}NPY, AIa^{13}NPY, GIy^{14}NPY, AIa^{15}NPY, AIa^{16}NPY, AIa^{17}NPY, AIa^{19}NPY, LyS^{19}NPY, AIa^{21}NPY, AIa^{22}NPY, LyS^{25}NPY, AIa^{26}NPY, Phe^{27}NPY, AIa^{28}NPY, GIn^{29}NPY, AIa^{30}NPY, AIa^{31}NPY, Phe^{36}NPY, HiS^{36}NPY, Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Pro^{13}Ala^{14}hPYY, hPP(l-7)-pNPY, hPP(l-17)-pNPY, Ty^NPY, AIa^{7}NPY or hPP(19-23)-pNPY.

In another embodiment, the PPF polypeptides of Formula I also do not include: Phe^{27}hPYY(3-36), Ile^{25}hPYY(3-36), Val^{28}hPYY(3-36), Gln^{29}hPYY(3-36), Val^{30}hPYY(3-36), Ile^{31}hPYY(3-36), Leu^{31}hPYY(3-36), Phe^{36}hPYY(3-36), Lys^{25}Phe^{27}hPYY(3-36), Lys^{25}Ile^{28}hPYY(3-36), Lys^{25}Val^{28}hPYY(3-36), Lys^{25}Gln^{29}hPYY(3-36), Lys^{25}Val^{30}hPYY(3-36), Lys^{25}Ile^{31}hPYY(3-36), Lys^{25}Leu^{31}hPYY(3-36), Lys^{25}Phe^{36}hPYY(3-36), Phe^{27}Ile^{28}hPYY(3-36), Phe^{27}Val^{28}hPYY(3-36), Phe^{27}Gln^{29}hPYY(3-36), Phe^{27}Val^{30}hPYY(3-36), Phe^{27}Ile^{31}hPYY(3-36), Phe^{27}Leu^{31}hPYY(3-36), Phe^{27}Phe^{36}hPYY(3-36), Gln^{29}Val^{30}hPYY(3-36), GIn^{29}IIe^{31}iPYY(S-So), Gln^{29}Leu^{31}hPYY(3-36), Gln^{29}Phe^{36}hPYY(3-36), Val^{30}Ile^{31}hPYY(3-36), Val^{30}Leu^{31}iPYY(S-So), Val^{30}Phe^{36}hPYY(3-36), or Leu^{31}Phe^{36}hPYY(3-36).

As will be recognized by one of skill in the art, the polypeptides of Formula I may be in the free acid form, or may be C-terminally amidated.

PYY Analog Polypeptides of the Present Invention

The PYY analog polypeptides of the present invention will generally include at least two PPF motifs including the N-terminal polyproline PPF motif and the C-terminal tail PPF motif, and will generally retain, at least in part, a biological activity of native human PYY, *e.g.*, the PYY analog polypeptides of the present invention will generally be PYY agonists. Moreover, the PYY analog polypeptide will have at least 50% sequence identity to PYY(3-36). In a preferred embodiment, the PYY analog polypeptides of the present invention will exhibit PYY activity in the treatment and prevention of metabolic conditions and disorders.

In one embodiment, the PYY analog polypeptides of the invention do not include any unnatural amino acid resides, and further with the provisio that the PYY analog polypeptides of the invention do not include any native PYY polypeptides or 1-4 N-terminal deletions thereof *(e.g.,* PYY(I-36), PYY(2-36), PYY(3-36)), PYY(4-36)). The PYY analog polypeptides of the invention also preferably do not include: PrO^{34}PYY, HiS^{34}PYY Lys^{25}hPYY(5-36), Arg^{4}hPYY(4-36), Gln^{4}hPYY(4-36), Asn^{4}hPYY(4-36), Lys^{25}hPYY(4-36), Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Tyr^{u} ^{6}pPYY, Pro^{13}Ala^{14}hPYY, Leu^{31}Pr$_{0}$^{34}PYY, FMS-PYY, FMS-PYY(3-36), Fmoc-PYY, Fmoc-PYY(3-36), FMS$_{2}$-PYY, FMS$_{2}$-PYY(3-36), Fmoc$_{2}$-PYY, or Fmoc$_{2}$-PYY(3-36).

In another embodiment, such PYY analog polypeptides of the invention also do not include: Thr^{27}hPYY(3-36), Ile^{30}hPYY(3-36), Ser^{32}hPYY(3-36), Lys^{33}hPYY(3-36), Asn^{34}hPYY(3-36), Lys^{35}hPYY(3-36), Thr^{36}hPYY(3-36), Lys^{25}Thr^{27}hPYY(3-36), Lys^{25}Ile^{30}hPYY(3-36), Lys^{25}Ser^{32}hPYY(3-36), Lys^{25}Lys^{33}hPYY(3-36), Lys^{25}Asn^{24}hPYY(3-36), Lys^{25}Lys^{35}hPYY(3-36), Lys^{25}Thr^{36}hPYY(3-36), Thr^{27}Ile^{28}hPYY(3-36), Thr^{27}Val^{28}hPYY(3-36), Thr^{27}Gln^{29}hPYY(3-36), Thr^{27}Ile^{30}hPYY(3-36), Thr^{27}Val^{30}hPYY(3-36), Thr^{27}Ile^{31}hPYY(3-36), Thr^{27}Leu^{31}liPYY(3-36), Thr^{27}Ser^{32}hPYY(3-36), Thr^{27}Lys^{33}hPYY(3-36), Thr^{27}Asn^{34}hPYY(3-36), Thr^{27}Lys^{35}hPYY(3-36), Thr^{27}Thr^{36}hPYY(3-36), Thr^{27}Phe^{36}hPYY(3-36), Phe^{27}Ile^{30}hPYY(3-36), Phe^{27}Ser^{32}hPYY(3-36), Phe^{27}Lys^{33}hPYY(3-36), Phe^{27}Asn^{34}hPYY(3-36), Phe^{27}Lys^{35}hPYY(3-36), Phe^{27}Thr^{36}hPYY(3-36), GIn^{29}Ile^{30}hPYY(3-36), Gln^{29}Ser^{32}hPYY(3-36), Gln^{29}Leu^{33}hPYY(3-36), Gln^{29}Asn^{34}hPYY(3-36), Gln^{29}Leu^{35}hPYY(3-36),

Gln^{29}Thr^{36}hPYY(3-36), He^{30}IIe^{31}IiPYY(3-36), Ile^{30}Leu^{31}hPYY(3-36), Ile^{30}Ser^{32}hPYY(3-36), Ile^{30}Lys^{33}hPYY(3-36), Ile^{30}Asn^{34}hPYY(3-36), Ile^{30}Lys^{35}hPYY(3-36), Ile^{30}Thr^{36}hPYY(3-36), Ile^{30}Phe^{36}hPYY(3-36), Val^{30}Ser^{32}hPYY(3-36), Val^{30}Lys^{33}hPYY(3-36), Val^{30}Asn^{34}hPYY(3-36), Val^{30}Lys^{35}hPYY(3-36), Val^{30}Thr^{36}hPYY(3-36) $_3$ He^{31}Ser^{32}IiPYY(3-36), He^{31}LyS^{33}IiPYY(S-SO), Ile^{31}Asn^{34}hPYY(3-36), He^{31}LyS^{35}IiPYY(3-36), lie^{31}ThT^{36}IiPYY(S-SO), Ile^{31}PIle^{36}hPYY(S-SO), Leu^{31}SeT^{3}VYY(S-SO), Leu^{31}LyS^{33}IiPYY(S-So), Leu^{31}ASn^{34}IiPYY(S-So), Leu^{31}Lys^{35}hPYY(3-36), Leu^{31}Thr^{36}hPYY(3-36), Ser^{32}Lys^{33}hPYY(3-36), Ser^{32}Asn^{34}hPYY(3-36), Ser^{32}Lys^{35}hPYY(3-36), Ser^{32}Thr^{36}hPYY(3-36), Ser^{32}Phe^{36}hPYY(3-36), Lys^{33}Asn^{34}hPYY(3-36), Lys^{33}Lys^{35}hPYY(3-36), Lys^{33}Thr^{36}hPYY(3-36), Lys^{33}Phe^{36}hPYY(3-36), Asn^{34}Lys^{35}hPYY(3-36), Asn^{34}Phe^{36}hPYY(3-36), Lys^{35}Thr^{36}IiPYY(3-36), Lys^{35}Phe^{36}hPYY(3-36), Thr^{27}liPYY(4-36), Phe^{27}hPYY(4-36), Ile^{28}hPYY(4-36), Val^{28}hPYY(4-36), Gln^{29}hPYY(4-36), Ile^{30}hPYY(4-36), Val^{30}hPYY(4-36), Ile^{31}hPYY(4-36), Leu^{31}hPYY(4-36), Ser^{32}hPYY(4-36), Lys^{33}hPYY(4-36), Asn^{34}hPYY(4-36), Lys^{35}hPYY(4-36), Thr^{36}hPYY(4-36), Phe^{36}hPYY(4-36), Lys^{25}Thr^{27}hPYY(4-36), Lys^{25}Phe^{27}hPYY(4-36), Lys^{25}Ile^{28}hPYY(4-36), Lys^{25}Val^{28}hPYY(4-36), Lys^{25}Gln^{29}hPYY(4-36), Lys^{25}Ile^{30}hPYY(4-36), Lys^{25}Val^{30}hPYY(4-36), Lys^{25}Ile^{31}hPYY(4-36), Lys^{25}Leu^{31}hPYY(4-36), Lys^{25}Ser^{32}hPYY(4-36), Lys^{25}Lys^{33}hPYY(4-36), Lys^{25}Asn^{24}hPYY(4-36), Lys^{25}Lys^{35}hPYY(4-36), Lys^{25}Thr^{36}hPYY(4-36), Lys^{25}Phe^{36}hPYY(4-36), Thr^{27}IIe^{2S}hPYY(4-36), Thr^{27}Val^{28}hPYY(4-36), Thr^{27}Gln^{29}hPYY(4-36), Thr^{27}Ile^{30}hPYY(4-36), Thr^{27}Val^{30}hPYY(4-36), ThT^{27}IIe^{31}IiPYY(4-36), Thr^{27}Leu^{31}hPYY(4-36), Thr^{27}Ser^{32}hPYY(4-36), Thr^{27}Lys^{33}hPYY(4-36), Thr^{27}Asn^{34}hPYY(4-36), Thr^{27}Lys^{35}hPYY(4-36), Thr^{27}Thr^{36}hPYY(4-36), Thr^{27}Phe^{36}hPYY(4-36), Phe^{27}Ile^{28}hPYY(4-36), Phe^{27}Val^{28}hPYY(4-36), Phe^{27}Gln^{29}hPYY(4-36), Phe^{27}Ile^{30}hPYY(4-36), Phe^{27}Val^{30}hPYY(4-36), Phe^{27}Ile^{31}hPYY(4-36), Phe^{27}Leu^{31}hPYY(4-36), Phe^{27}Ser^{32}hPYY(4-36), Phe^{27}Lys^{33}hPYY(4-36), Phe^{27}Asn^{34}hPYY(4-36), Phe^{27}Lys^{35}hPYY(4-36), Phe^{27}Thr^{36}hPYY(4-36), Phe^{27}Phe^{36}hPYY(4-36), GIn^{29}IIe^{30}IiPYY(4-36), GIn^{29}VaI^{30}IiPYY(4-36), GIn^{29}IIe^{31}IiPYY(4-36), Gln^{29}Leu^{31}hPYY(4-36), Gln^{29}Ser^{32}hPYY(4-36), Gln^{29}Leu^{33}hPYY(4-36), Gln^{29}Asn^{34}hPYY(4-36), Gln^{29}Leu^{35}hPYY(4-36), Gln^{29}Thr^{36}hPYY(4-36),

Gln^{29}Phe^{36}hPYY(4-36), Ile^{30}Ile^{31}hPYY(4-36), Ile^{30}Leu^{31}hPYY(4-36), Ile^{30}Ser^{32}hPYY(4-36), Ile^{30}Lys^{33}hPYY(4-36), Ile^{30}Asn^{34}hPYY(4-36), Ile^{30}Lys^{35}hPYY(4-36), Ile^{30}Thr^{36}hPYY(4-36), Ile^{30}Phe^{36}hPYY(4-36), Val^{30}Ile^{31}IiPYY(4-36), Val^{30}LeU^{31}IiPYY(4-36), Val^{30}Ser^{32}hPYY(4-36), Val^{30}Lys^{33}hPYY(4-36), Val^{30}Asn^{34}hPYY(4-36), Val^{30}Lys^{35}hPYY(4-36), Val^{30}Thr^{36}hPYY(4-36), Val^{30}Phe^{36}hPYY(4-36), lie^{31}Ser^{32}IiPYY(4-36), He^{31}LyS^{33}IiPYY(4-36), Ile^{31}Asn^{34}hPYY(4-36), Ile^{31}Lys^{35}hPYY(4-36), He^{31}ThT^{36}IiPYY(4-36), Leu^{31}PIle^{36}IiPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Ser^{32}liPYY(4-36), Val^{31}LyS^{33}IiPYY(4-36), Leu^{31}Asn^{34}hPYY(4-36), Leu^{31}LyS^{35}IiPYY(4-36), Leu^{31}Thr^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Ser^{32}Lys^{33}hPYY(4-36), Ser^{32}Asn^{34}hPYY(4-36), Ser^{32}Lys^{35}hPYY(4-36), Ser^{32}Thr^{36}hPYY(4-36), Ser^{32}Phe^{36}hPYY(4-36), Lys^{33}Asn^{34}hPYY(4-36), Lys^{33}Lys^{35}hPYY(4-36), Lys^{33}Thr^{36}hPYY(4-36), Lys^{33}Phe^{36}hPYY(4-36), Asn^{34}Lys^{35}hPYY(4-36), Asn^{34}Phe^{36}hPYY(4-36), Lys^{35}Thr^{36}hPYY(4-36), Lys^{35}Phe^{36}hPYY(4-36), Thr^{27}liPYY(5-36), Phe^{27}hPYY(5-36), He^{28}hPYY(5-36), Val^{28}hPYY(5-36), Gln^{29}hPYY(5-36), Ile^{30}hPYY(5-36), Val^{30}hPYY(5-36), lie^{31}IiPYY(S-SO), Leu^{31}IiPYY(S-SO), Ser^{32}hPYY(5-36), Lys^{33}hPYY(5-36), Asn^{34}hPYY(5-36), Lys^{35}hPYY(5-36), Thr^{36}hPYY(5-36), Phe^{36}hPYY(5-36), Lys^{25}Thr^{27}hPYY(5-36), Lys^{25}Phe^{27}hPYY(5-36), Lys^{25}Ile^{28}hPYY(5-36), Lys^{25}Val^{28}hPYY(5-36), Lys^{25}Gln^{29}hPYY(5-36), Lys^{25}Ile^{30}hPYY(5-36), Lys^{25}Val^{30}hPYY(5-36), LyS^{25}Ile^{31}IiPYY(S-So), Lys^{25}Leu^{3}VYY(5-36), Lys^{25}Ser^{32}hPYY(5-36), Lys^{25}Lys^{33}hPYY(5-36), Lys^{25}Asn^{24}hPYY(5-36), Lys^{25}Lys^{35}hPYY(5-36), Lys^{25}Thr^{36}liPYY(5-36), Lys^{25}Phe^{36}hPYY(5-36), Thr^{27}Ue^{28}hPYY(5-36), Thr^{27}Val^{28}hPYY(5-36), Thr^{27}Gln^{29}hPYY(5-36), Thr^{27}Ile^{30}hPYY(5-36), Thr^{27}Val^{30}hPYY(5-36), ThT^{27}Ile^{3}VYY(S-So), Thr^{27}Leu^{31}IiPYY(S-So), Thr^{27}Ser^{32}hPYY(5-36)$_3$ Thr^{27}Lys^{33}hPYY(5-36), Thr^{27}Asñ^{34}hPYY(5-36), Tlir^{27}Lys^{35}hPYY(5-36)$_5$ Thr^{27}Thr^{36}hPYY(5-36), Thr^{27}Phe^{36}hPYY(5-36), Phe^{27}Ile^{28}hPYY(5-36), Phe^{27}Val^{28}hPYY(5-36), Phe^{27}Gln^{29}hPYY(5-36), Phe^{27}Ile^{30}hPYY(5-36), Phe^{27}Val^{30}hPYY(5-36), Phe^{27}lle^{31}IiPYY(S-SO), Phe^{27}Leu^{31}IiPYY(S-So), Phe^{27}Ser^{32}hPYY(5-36), Phe^{27}Lys^{33}hPYY(5-36), Phe^{27}Asn^{34}hPYY(5-36), Phe^{27}Lys^{35}hPYY(5-36), Phe^{27}Thr^{36}hPYY(5-36), Phe^{27}Phe^{36}hPYY(5-36), Gln^{29}Ile^{30}hPYY(5-36), Gln^{29}Val^{30}hPYY(5-36), GIn^{29}Ile^{31}IiPYY(5-36), Gln^{29}Leu^{31}hPYY(5-36),

Gln^{29}Ser^{32},hPYY(5-36), Gln^{29}Leu^{33}hPYY(5-36), Gln^{29}Asn^{34}hPYY(5-36), Gln^{29}Leu^{35}hPYY(5-36), Gln^{29}Thr^{36}hPYY(5-36), Gln^{29}Phe^{36}hPYY(5-36), He^{30}IIe^{31}IiPYY(5-36), He^{30}LeU^{31}IiPYY(S-SO), Ile^{30}Ser^{32}hPYY(5-36), Ile^{30}Lys^{33}hPYY(5-36), Ile^{30}Asn^{34}hPYY(5-36), Ile^{30}Lys^{35}hPYY(5-36), He^{30}ThT^{36}IiPYY(5-36), Ile^{30}Phe^{36}hPYY(5-36), VaI^{30}IIe^{31}IiPYY(S-So), VaI^{30}LeU^{31}IiPYY(S-SO), Val^{30}Ser^{32}hPYY(5-36), Val^{30}Lys^{33}hPYY(5-36), Val^{30}Asn^{34}hPYY(5-36), Val^{30}Lys^{35}hPYY(5-36), Val^{30}Thr^{36}hPYY(5-36), Val^{30}Phe^{36}hPYY(5-36), Ile^{31}Ser^{32}hPYY(5-36), Ile^{31}Lys^{33}hPYY(S-SO), He^{31}As$_{n}$^{34}IiPYY(S-SO), Ile^{31}Lys^{35}hPYY(5-36), Ile^{31}Thr^{36}hPYY(5-36), Leu^{31}PIle^{36}IiPYY(S-SO), Leu^{31}Phe^{36}hPYY(5-36), Leu^{31}Ser^{32}hPYY(5-36), Val^{31}Lys^{33}hPYY(5-36), Leu^{31}Asn^{34}hPYY(5-36), Leu^{31}Lys^{35}hPYY(5-36), Leu^{31}Tlir^{36}hPYY(5-36), Leu^{31}Phe^{36}hPYY(5-36), Ser^{32}Lys^{33}hPYY(5-36), Ser^{32}Asn^{34}hPYY(5-36), Ser^{32}Lys^{35}hPYY(5-36), Ser^{32}Tlir^{36}hPYY(5-36), Ser^{32}Phe^{36}hPYY(5-36), Lys^{33}Asn^{34}hPYY(5-36), Lys^{33}Lys^{35}hPYY(5-36), Lys^{33}Thr^{36}liPYY(5-36), Lys^{33}Phe^{36}hPYY(5-36), Asn^{34}Lys^{35}hPYY(5-36), Asn^{34}Phe^{36}hPYY(5-36), Lys^{35}Thr^{36}hPYY(5-36), or Lys^{35}Phe^{36}liPYY(5-36).

In another embodiment, the PYY analog polypeptides of the invention do not include any unnatural amino acid residues, and preferably comprise a C-terminal tail motif of hPYY. The C-terminal motif may preferably comprise amino acid residues 32-35 of hPYY, *e.g.*, Thr, Arg, GIn, Arg (SEQ ID NO: 351). In such an embodiment, the PYY analog polypeptides of the invention do not include any native PYY polypeptides or 1-4 N-terminal deletions thereof *(e.g.,* PYY(I -36), PYY(2-36), PYY(3-36) and, PYY(4-36)). Such PYY analogs also preferably do not include: Lys^{25}hPYY(5-36), Arg^{4}hPYY(4-36), GIn^{4}IiPYY(4-36), Asn^{4}hPYY(4-36), Lys^{25}hPYY(4-36), Leu^{3}hPYY(3-36), Va^{3}hPYY(3-36), Lys^{25} PYY(3-36), Tyr$^{1-36}$pPYY, Pro^{13}Ala^{14}hPYY, FMS-PYY, FMS-PYY(3-36), Fmoc-PYY, Fmoc-PYY(3-36), FMS$_{2}$-PYY, FMS$_{2}$-PYY(3-36), Fmoc$_{2}$-PYY, or Fmoc$_{2}$-PYY(3-36).

In another aspect, such PYY analog polypeptides of the invention comprising a C-terminal tail motif of hPYY also do not include: Thr^{27}hPYY(3-36), Ile^{30}hPYY(3-36), Thr^{36}hPYY(3-36), Lys^{25}Thr^{27}hPYY(3-36), Lys^{25}Ue^{30}hPYY(3-36), Lys^{25}Asn^{24}hPYY(3-36), Lys^{25}Thr^{36}hPYY(3-36), Thr^{27}Ile^{28}hPYY(3-36), Thr^{27}Val^{28}hPYY(3-36), Thr^{27}Gln^{29}hPYY(3-36), Thr^{27}Ile^{30}hPYY(3-36),

Thr^{27}Val^{30}hPYY(3-36), ThT^{27}IIe^{31}IiPYY(S-So), Thr^{27}Leu^{31}IiPYY(S-SO), Thr^{27}Thr^{36}hPYY(3-36), Thr^{27}Phe^{36}hPYY(3-36), Phe^{27}Ile^{30}hPYY(3-36), Phe^{27}Thr^{36}hPYY(3-36), Gln^{29}Ile^{30}hPYY(3-36), Gln^{29}Thr^{36}hPYY(3-36), Ile^{30}Ile^{31}hPYY(3-36), He^{30}Leu^{31}hPYY(3-36), Ile^{30}Thr^{36}hPYY(3-36), Ile^{30}Phe^{36}hPYY(3-36), Val^{30}Thr^{36}hPYY(3-36), Ile^{31}Thr^{36}hPYY(3-36), He^{31}Phe^{36}IiPYY(S-So), Leu^{31}Thr^{36}hPYY(3-36), Thr^{27}hPYY(4-36), Phe^{27}hPYY(4-36), Ile^{28}hPYY(4-36), Val^{28}hPYY(4-36), Gln^{29}hPYY(4-36), Ile^{30}hPYY(4-36), Val^{30}hPYY(4-36), Ile^{31}hPYY(4-36), Leu^{31}IiPYY(4-36), Thr^{36}hPYY(4-36), Phe^{36}hPYY(4-36), Lys^{25}Thr^{27}hPYY(4-36), Lys^{25}Phe^{27}hPYY(4-36), Lys^{25}Ile^{28}hPYY(4-36), Lys^{25}Val^{28}hPYY(4-36), Lys^{25}Gln^{29}hPYY(4-36), Lys^{25}Ile^{30}hPYY(4-36), Lys^{25}Val^{30}hPYY(4-36), Lys^{25}Ile^{31}hPYY(4-36), Lys^{25}Leu^{31}hPYY(4-36), Lys^{25}Thr^{36}hPYY(4-36), Lys^{25}Phe^{36}hPYY(4-36), Thr^{27}Ile^{28}hPYY(4-36), ThT^{27}VaI^{28}IiPYY(4-36), Thr^{27}Gln^{29}hPYY(4-36), Thr^{27}Ile^{30}hPYY(4-36), Thr^{27}Val^{30}hPYY(4-36)$_5$ Thr^{27}Ile^{31}hPYY(4-36), Thr^{27}Leu^{31}hPYY(4-36), Tlir^{27}Thr^{36}hPYY(4-36), Thr^{27}Phe^{36}hPYY(4-36), Phe^{27}Ile^{28}hPYY(4-36), Phe^{27}Val^{28}hPYY(4-36), Phe^{27}Gln^{29}IiPYY(4-36), Phe^{27}Ile^{30}hPYY(4-36), Phe^{27}Val^{30}hPYY(4-36), Phe^{27}Ile^{31}IiPYY(4-36), Phe^{27}Leu^{31}hPYY(4-36), Phe^{27}Thr^{36}hPYY(4-36), Phe^{27}Phe^{36}hPYY(4-36), GIn^{29}IIe^{30}IiPYY(4-36), Ghr^{29}Val^{30}hPYY(4-36), GIn^{29}IIe^{31}IiPYY(4-36), Gln^{29}Leu^{31}hPYY(4-36), Gln^{29}Thr^{36}hPYY(4-36), Gln^{29}Phe^{36}hPYY(4-36), He^{30}IIe^{31}IiPYY(4-36), He^{30}LeU^{31}IiPYY(4-36), Ile^{30}Thr^{36}hPYY(4-36), Ile^{30}Plie^{36}hPYY(4-36), VaI^{30}IIe^{31}IiPYY(4-36), Val^{30}Leu^{31}hPYY(4-36), VaI^{30}ThT^{36}IiPYY(4-36), Val^{30}Phe^{36}hPYY(4-36), Ile^{31}Thr^{36}hPYY(4-36), Leu^{31}PhC^{36}IiPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Leu^{31}Thr^{36}hPYY(4-36), Leu^{31}Phe^{36}hPYY(4-36), Thr^{27}hPYY(5-36), Phe^{27}hPYY(5-36), Ile^{28}hPYY(5-36), Val^{28}hPYY(5-36), Gln^{29}hPYY(5-36), Ile^{30}hPYY(5-36), Val^{30}hPYY(5-36), Ile^{31}hPYY(5-36), Leu^{31}hPYY(5-36), Thr^{36}hPYY(5-36), Phe^{36}hPYY(5-36), Lys^{25}Thr^{27}hPYY(5-36), Lys^{25}Phe^{27}hPYY(5-36), Lys^{25}Ile^{28}hPYY(5-36), Lys^{25}Val^{28}hPYY(5-36), Lys^{25}Gln^{29}hPYY(5-36), Lys^{25}Ile^{30}hPYY(5-36), Lys^{25}Val^{30}hPYY(5-36), LyS^{25}IIe^{31}IiPYY(S-SO), Lys^{25}Leu^{31}hPYY(5-36), Lys^{25}Thr^{36}liPYY(5-36), Lys^{25}Phe^{36}hPYY(5-36), Thr^{27}Ile^{28}hPYY(5-36), Thr^{27}Val^{28}hPYY(5-36), Thr^{27}Gln^{29}hPYY(5-36), Thr^{27}Ile^{30}hPYY(5-36), Thr^{27}Val^{30}hPYY(5-36), Thr^{27}Ile^{31}hPYY(5-36), Thr^{27}Leu^{31}hPYY(5-36),

Thr^{27}Thr^{36}hPYY(5-36), Thr^{27}Phe^{36}hPYY(5-36), Phe^{27}Ile^{28}hPYY(5-36), Phe^{27}Val^{28}hPYY(5-36), Phe^{27}Gln^{29}hPYY(5-36), Phe^{27}Ile^{30}hPYY(5-36), Phe^{27}Val^{30}hPYY(5-36), Phe^{27}Ile^{31}IiPYY(S-SO), Phe^{27}Leu^{31}IiPYY(S-SO), Phe^{27}Thr^{36}hPYY(5-36), Phe^{27}Phe^{36}hPYY(5-36), Gln^{29}Ile^{30}hPYY(5-36), Gln^{29}Val^{30}hPYY(5-36), GIn^{29}IIe^{31}IiPYY(S-SO)$_5$ Gln^{29}Leu^{31}hPYY(5-36), Gln^{29}Thr^{36}hPYY(5-36), Gln^{29}Phe^{36}hPYY(5-36), He^{30}IIe^{31}IiPYY(S-So), Ile^{30}Leu^{31}hPYY(5-36), Ile^{30}Thr^{36}hPYY(5-36), Ile^{30}Phe^{36}hPYY(5-36), Val^{30}Ile^{31}hPYY(5-36), VaI^{30}LeU^{31}IiPYY(S-SO), Val^{30}Thr^{36}hPYY(5-36), Val^{30}Phe^{36}hPYY(5-36), Ile^{31}Thr^{36}hPYY(5-36), Leu^{31}PIle^{36}IiPYY(S-SO), Leu^{3I}Phe^{36}hPYY(5-36), Leu^{31}ThT^{36}IiPYY(S-SO), or Leu^{31}Phe^{36}hPYY(5-36).

The PYY analog polypeptides of the invention are also preferably at least 34 amino acids in length. Further, in a preferred embodiment, the PYY analog polypeptides of the invention include only natural L amino acid residues and/or modified natural L amino acid residues. Alternatively, in a preferred embodiment, the PYY analog polypeptides of the invention do not include unnatural amino acid residues.

More particularly, in one aspect, the present invention relates to PYY analog polypeptides including one or more amino acid sequence modifications. Such modifications include substitutions, insertions, and/or deletions, alone or in combination. In a preferred aspect, the PYY analog polypeptides of the invention include one or more modifications of a "non-essential" amino acid residue. In the context of the invention, a "non-essential" amino acid residue is a residue that can be altered, *i.e.*, deleted or substituted, in the native human PYY amino acid sequence without abolishing or substantially reducing the PYY agonist activity of the PYY analog polypeptide. Preferably, the PYY analog polypeptides of the invention retain at least about 25%, preferably about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% percent of the biological activity of native human PYY with regard to the reduction of nutrient availability. In another embodiment, the PYY analog polypeptides of the invention exhibit improved PYY agonist activity. Preferably, the PYY analog polypeptides of the invention exhibits at least about 110%, 125%, 130%, 140%, 150%, 200%, or more of the biological activity of native human PYY with regard to the reduction of nutrient availability.

Preferred PYY analog polypeptide are those having a potency in one of the assays described herein (preferably food intake, gastric emptying, pancreatic secretion, or weight reduction assays) which is equal to or greater than the potency of NPY, PYY, or PYY(3-36) in that same assay. Alternatively, preferred PYY analog polypeptides of the invention may exhibit improved ease of manufacture, stability, and/or ease of formulation, as compared to PP, NPY, PYY, or PYY(3-36).

Substitutions

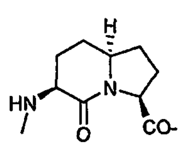
In one embodiment, the PYY analog polypeptides of the invention may have one or more substitutions in the amino acid sequence of native human PYY (SEQ ID NO: 2), alone or in combination with one or more insertions or deletions. Preferably, the substitution does not abolish or substantially reduce the PYY agonist activity of the PYY analog polypeptide. In one aspect, the present invention relates to PYY analog polypeptides that have a single substitution, or consecutive or non-consecutive substitution of more than one amino acid residues in the amino acid sequence of native human PYY (SEQ ID NO: 2). Preferably, the PYY analog polypeptides of the invention include one, two, or three amino acid substitutions.

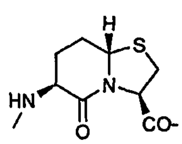
Preferably, the amino acid residues of native human PYY (SEQ ID NO: 2) at the helical C-terminus region of PYY (e.g., residues 20, 24, 25, 27 and 29), the tail end residues (32-36), and/or the N-terminus prolines at position 5 and 8 are not substituted. In a preferred embodiment, amino acid residues are not substituted at positions 32 through 36 of native human PYY (SEQ ID NO: 2). In another embodiment, amino acid residues of native human PYY (SEQ ID NO: 2) are not substituted at one or more amino acid sequence positions selected from: 5, 7, 8, 20, 24, 25, 27, 29, 32, 33, 34, 35, 36, and any combination thereof.

Preferred substitutions include conserved amino acid substitutions. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain, or physicochemical characteristics (*e.g.*, electrostatic, hydrogen bonding, isosteric, hydrophobic features). Families of amino acid residues having similar side chains are known in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*,

glycine, asparagine, glutamine, serine, threonine, tyrosine, methionine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan), β-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

In another embodiment, the PYY analog polypeptides of the invention may include substitutions of one or more unnatural and/or non-amino acids, *e.g.*, amino acid mimetics, into the sequence of PYY (SEQ ID NO: 2). In a preferred embodiment, the non-amino acids inserted into the sequence of PYY (SEQ ID NO: 2) may be β-turn mimetics or linker molecules, such as -NH-X-CO-, wherein X = $(CH_2)_n$ (where n can be 2-20) or -NH-CH_2CH_2(-O-CH_2CH_2-O-$)_m$-CH_2-CO- (where m = 1-5). Preferred linker molecules include aminocaproyl ("Aca"), β-alanyl, and 8-amino-3,6-dioxaoctanoyl. β-turn mimetics are available commercially (BioQuadrant Inc, Quebec, Canada) and have been described in literature (Hanessian *et ah,* Tetrahedron 12789-854 (1997); Gu *et ah,* Tetrahedron Letters 44: 5863-6 (2003); Bourguet et al., Bioorganic & Medicinal Chemistry Letters 13: 1561-4 (2003); Grieco *et ah,* Tetrahedron Letters 43: 6297-9 (2002); Souers *et ah,* Tetrahedron 57: 7431-48 (2001); Tsai *et ah,* Bioorganic & Medicinal Chemistry 7: 29-38 (1999); Virgilio *et ah,* Tetrahedron 53: 6635-44 (1997)). Preferred β-turn mimetics include mimic A and mimic B illustrated below.


mimic A


mimic B

Preferred PYY analog polypeptides comprising amino acid sequence β-turn mimetic substitutions include native human PYY (SEQ ID NO: 2), wherein amino acids at positions x and x+1 are substituted with β-turn mimetics selected from the group consisting of mimic A and mimic B, wherein x is selected from the amino acids at amino acid positions 8 to 14 of native human PYY. Altenatively, known dipeptide turn inducers may be susbtituted, for example, Ala-Aib and Ala-Pro dipeptides.

Other preferred PYY analog polypeptides comprising amino acid sequence substitutions include the PYY analog polypeptides of the Formula (II) (SEQ ID NO: 88):

Xaat Xaa_2 Xaa_3 Xaa4 Pro Xaa_6 Xaa_7 Pro Xaag Xaa_{10}
Xaaπ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30}
Xaa_3i Thr Arg Gln Arg Xaa_{36}

wherein:

Xaa_1 is Tyr, Ala, Phe, Trp, or absent;
Xaa_2 is Pro, Gly, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is He, Ala, NorVal, Val, Leu, Pro, Ser or Thr;
Xaa_4 is Lys, Ala, Gly, Arg, d-Ala, homoLys, homoArg, Glu, or Asp;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaag is Gly, Ala Ser, sarcosine, Pro, or Aib;
Xaaio is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa] *i* is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, or homoSer;
Xaa_{14} is Pro, Ala, homo-Pro, hydroxy-Pro, Aib, or Gly;
Xaa_{15} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa_{16} is Glu, Ala, Asp, Asn, or Gln;
Xaa_{17} is Leu, Ala, Met, Trp, He, Val, or NorVal;
Xaa_{18} is Asn, Asp, Ala, Glu, Gln, Ser or Thr;
Xaaip is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, He, Val, or Ala;
Xaa_{30} is Leu, Ala, NorVal, Val, lie, or Met;
Xaa_{31} is Ala, Val, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, PYY(2-36), PP(2-36), Ala^{13}NPY, Leu^3hPYY(3-36), Val^3hPYY(3-36), hPP(l-7)-pNPY, or hPP(l-17)-pNPY.

In another embodiment, the PYY analog polypeptides of Formula II also do not include: Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), Val^{30}hPYY(3-36), Ile^{31}hPYY(3-36), Leu^{31}IiPYYp-SO), Phe^{36}hPYY(3-36), $Val^{30}Ile^{31}$IiPYY(3-36), $Val^{30}LeU^{31}$IiPYY(S-SO), $Val^{30}Phe^{36}$hPYY(3-36), or $Leu^{31}Phe^{36}$hPYY(3-36).

As will be recognized by one of skill in the art, the polypeptides of Formula II may be in the free acid form, or may be C-terminally amidated.

Other preferred PYY analog polypeptides comprising amino acid sequence substitutions include the PYY analog polypeptides of the Formula (III) (SEQ ID NO: 348):

Xaa! Xaa_2 Xaa_3 Xaa4 Pro Xaa_6 Xaa_7 Pro Xaap Xaaio
Xaaπ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30}
Xaa_{31} Thr Arg GIn Arg Xaa_{36}
wherein:
Xaa_1 is Tyr, Phe, Trp, or absent;
Xaa_2 is Pro, GIy, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is lie, Ala, NorVal, VaI, Leu, Pro, Ser or Thr;
Xaa_4 is Lys, Ala, GIy, Arg, d-Ala, homoLys, homoArg, GIu, or Asp;
Xaa_6 is GIu, Ala, VaI, Asp, Asn, or GIn;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaa_9 is GIy, Ala Ser, sarcosine, Pro, or Aib;
Xaa_{10} is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaai *i* is Asp, Ala, GIu, Asn, GIn, Pro, Aib, or GIy;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, Pro, or homoSer;
Xaa_{14} is Pro, Ala, homo-Pro, hydroxyPro, Aib, or GIy;
Xaais is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaa_{16} is GIu, Ala, Asp, Asn, or GIn;
Xaa_{17} is Leu, Ala, Met, Trp, He, VaI, or NorVal;
Xaa_{18} is Asn, Asp, Ala, GIu, GIn, Ser or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, GIn, or N(Me)AIa;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, He, VaI, or Ala;
Xaa_{30} is Leu, Ala, NorVal, VaI, lie, or Met;
Xaa_{31} is Ala, VaI, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, NPY(2-36), PYY(2-36), PP(2-36), AIa^3NPY, AIa^4NPY, AIa^6NPY, AIa^7NPY, Tyr^7pNPY, AIa^9NPY, $AIa^{10}NPY$, $AIa^{11}NPY$, $AIa^{13}NPY$, $GIy^{14}NPY$, $AIa^{15}NPY$, $AIa^{16}NPY$, $AIa^{17}NPY$, $AIa^{19}NPY$, $LyS^{19}NPY$, $AIa^{21}NPY$, $AIa^{22}NPY$, $LyS^{25}NPY_5$ $AIa^{26}NPY$, $Phe^{27}NPY$, $AIa^{28}NPY$, $GIn^{29}NPY$, $AIa^{30}NPY$, $AIa^{31}NPY$, $Phe^{36}NPY$, $HiS^{36}NPY$, $Leu^3hPYY(3-36)$, $Val^3hPYY(3-36)$, $Lys^{25}hPYY(3-36)$, $Pro^{13}Ala^{14}hPYY$, TyT^1NPY, AIa^7NPY, or hPP(19-23)-pNPY.

In another embodiment, the PYY analog polypeptides of Formula III also do not include: Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), $Val^{3 q}$hPYY(3-36), $lie^{3 1}$IiPYY(S-SO), $Leu^{3 1}$hPYY(3-36), Phe^{36}hPYY(3-36), $Val^{30}Ile^{3 1}$hPYY(3-36), $Val^{30}Leu^{3 1}$hPYY(3-36), $Val^{30}Phe^{36}$hPYY(3-36), or $Leu^{3 1}Phe^{36}$IiPYY(S-36).

As will be recognized by one of skill in the art, the polypeptides of Formula III may be in the free acid form, or may be C-terminally amidated.

Other preferred PYY analog polypeptides comprising amino acid sequence substitutions include the PYY analog polypeptides of the Formula (IV) (SEQ ID NO: 349):

Xaaj Xaa_2 Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaa_9 Xaa_{10}
Xaaπ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30}
Xaa_{31} Tlir Arg GIn Arg Xaa_{36}
wherein:
Xaai is Tyr, Phe, Trp, or absent;
Xaa_2 is Pro, GIy, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is He, Ala, NorVal, VaI, Leu, Pro, Ser or Thr;
Xaa_4 is Lys, Ala, GIy, Arg, d-Ala, homoLys, homoArg, GIu, or Asp;
Xaa_6 is GIu, Ala, VaI, Asp, Asn, or GIn;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaa_9 is GIy, Ala Ser, sarcosine, Pro, or Aib;
Xaaio is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaaπ is Asp, Ala, GIu, Asn, GIn, Pro, Aib, or GIy;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, or homoSer;
$Xaaj_4$ is Pro, Ala, homo-Pro, hydroxyPro, Aib, or GIy;
Xaa_{15} is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaa_{16} is GIu, Ala, Asp, Asn, or GIn;
Xaa_{17} is Leu, Ala, Met, Trp, He, VaI, or NorVal;
Xaa_{18} is Asn, Asp, Ala, GIu, GIn, Ser or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, GIn, or N(Me)AIa;
Xaa_2i is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, He, VaI, or Ala;
Xaa_{30} is Leu, Ala, NorVal, VaI, He, or Met;
Xaa_3i is Ala, VaI, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, PYY(2-36), AIa^{13}NPY, Leu^3hPYY(3-36), or Val^3hPYY(3-36).

In another embodiment, the PYY analog polypeptides of Formula IV also do not include: Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), Val^{30}hPYY(3-36), He^{31}IiPYY(S-SO), Leu^{31}IiPYY(S-SO), Phe^{36}hPYY(3-36), $Val^{30}Ile^{31}$hPYY(3-36), $Val^{30}Leu^{31}$hPYY(3-36), $Val^{30}Phe^{36}$liPYY(3-36), or $Leu^{31}Phe^{36}$hPYY(3-36).

As will be recognized by one of skill in the art, the polypeptides of Formula IV may be in the free acid form, or may be C-terminally amidated.

Other preferred PYY analog polypeptides comprising amino acid sequence linker substitutions include PYY(I -4)Aminocaproyl(14-36) (IUPAC [Aca^{5-13}]PYY) (Aminocaproyl is abbreviated as "Aca"), PYY(l-4)Aca(15-36), PYY(l-4)Aca(16-36), PYY(I -4)Aca(22-36) (IUPAC [Aca^{5-21}]PYY), and PYY(I -4)Aca(25-36) (IUPAC [Aca^{5-24}]PYY) (SEQ ID NOS: 180-184).

Deletions and Truncations

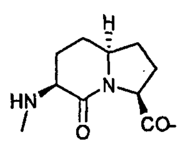
In another embodiment, the PYY analog polypeptides of the invention may have one or more amino acid residues deleted from the amino acid sequence of native human PYY (SEQ ID NO: 2), alone or in combination with one or more insertions or substitutions. In one aspect, the PYY analog polypeptides of the invention may have one or more amino acid residues deleted from the N-terminus or C-terminus of native human PYY (SEQ ID NO: 2), with the proviso that the polypeptide is not SEQ ID NO: 3. In another embodiment, the PYY analog polypeptides of the invention may have one or more amino acid residues deleted at amino acid positions 2 through 35 of native human PYY (SEQ ID NO: 2). Such deletions may include more than one consecutive or non-consecutive deletions at amino acid positions 2 through 35 of native human PYY (SEQ ID NO: 2). In a preferred embodiment, the amino acid residues at positions 24 through 36 of native human PYY (SEQ ID NO: 2) are not deleted.

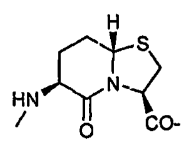
In another embodiment, the PPF polypeptides of the invention described in Formulas I to V *(infra* and *supra)* may include N or C-terminal truncations, or internal deletions at amino acid positions 2 to 35 of Formula I, II, III, IV, or V, so long as at least one biological activity of a native PPF polypeptide is retained. In preferred embodiments, the amino acid residues at positions 5 through 8 and 24 through 36, more preferably 5 through 8 and 32 through 35 are not deleted.

Insertions

In another embodiment, the PYY analog polypeptides of the invention may have one or more amino acid residues inserted into the amino acid sequence of native human PYY (SEQ ID NO: 2), alone or in combination with one or more deletions and/or substitutions. In one aspect, the present invention relates to PYY analog polypeptides that have a single insertion, or consecutive or non-consecutive insertions of more than one amino acid residues into the amino acid sequence of native human PYY (SEQ ID NO: 2). In a preferred embodiment, amino acid residues are not inserted at positions 24 through 36 of native human PYY (SEQ ID NO: 2).

In another embodiment, the PYY analog polypeptides of the invention may include insertions of one or more unnatural amino acids and/or non-amino acids into the sequence of PYY (SEQ ID NO: 2). In a preferred embodiment, the unnatural amino acids inserted into the sequence of PYY (SEQ ID NO: 2) may be β-turn mimetics or linker molecules. Preferred linker molecules include aminocaproyl ("Aca"), β-alanyl, and 8-amino-3,6-dioxaoctanoyl. Preferred β-turn mimetics include mimic A and mimic B illustrated below, also Ala-Aib and Ala-Pro dipeptides.


mimic A


mimic B

In another embodiment, PYY analog polypeptides of the invention may include insertions of polyamino acid sequences (*e.g.*, poly-his, poly-arg, poly-lys, poly-ala, etc.) at either terminus of the polypeptide, known as "extensions" or "tails."

Preferred PYY analog polypeptides comprising amino acid sequence insertions include alanine substitutions at each amino acid position along the length of native human PYY. Such PYY analog polypeptides include PYY (+Axa), wherein x is selected from 1' to 36 (SEQ ID NOS: 54-87).

Derivatives

The present invention also relates to derivatives of the PYY analog polypeptides of the invention. Such derivatives include PYY analog polypeptides conjugated to one or more water soluble polymer molecules, such as polyethylene glycol ("PEG") or

fatty acid chains of various lengths *(e.g.,* stearyl, palmitoyl, octanoyl), by the addition of polyamino acids, such as poly-his, poly-arg, poly-lys, and poly-ala, or by addition of small molecule substituents include short alkyls and constrained alkyls *(e.g.,* branched, cyclic, fused, adamantyl), and aromatic groups. The water soluble polymer molecules will preferably have a molecular weight ranging from about 500 to about 20,000 Daltons.

Such polymer-conjugations may occur singularly at the N- or C-terminus or at the side chains of amino acid residues within the sequence of the PYY analog polypeptides. Alternatively, there may be multiple sites of derivatization along the PYY analog polypeptide. Substitution of one or more amino acids with lysine, aspartic acid, glutamic acid, or cysteine may provide additional sites for derivatization. *See, e.g.,* U.S. Patent Nos. 5,824,784 and 5,824,778. Preferably, the PYY analog polypeptides may be conjugated to one, two, or three polymer molecules.

The water soluble polymer molecules are preferably linked to an amino, carboxyl, or thiol group, and may be linked by N or C termini, or at the side chains of lysine, aspartic acid, glutamic acid, or cysteine. Alternatively, the water soluble polymer molecules may be linked with diamine and dicarboxylic groups. In a preferred embodiment, the PYY analog polypeptides of the invention are conjugated to one, two, or three PEG molecules through an epsilon amino group on a lysine amino acid.

PYY analog polypeptide derivatives of the invention also include PYY analog polypeptides with chemical alterations to one or more amino acid residues. Such chemical alterations include amidation, glycosylation, acylation, sulfation, phosphorylation, acetylation, and cyclization. The chemical alterations may occur singularly at the N- or C-terminus or at the side chains of amino acid residues within the sequence of the PYY analog polypeptides. In one embodiment, the C-terminus of these peptides may have a free -OH or $-NH_2$ group. In another embodiment, the N-terminal end may be capped with an isobutyloxycarbonyl group, an isopropyloxycarbonyl group, an n-butyloxycarbonyl group, an ethoxycarbonyl group, an isocaproyl group (isocap), an octanyl group, an octyl glycine group (G(Oct)), an 8-aminooctanic acid group or a Fmoc group. In a preferred embodiment, cyclization can be through the formation of disulfide bridges, *see, e.g.,* SEQ ID NO. 171.

Alternatively, there may be multiple sites of chemical alteration along the PYY analog polypeptide.

Preferred Analogs and Derivatives

In a preferred aspect of the invention, the PYY analog polypeptides include combinations of the above-described modifications, *i.e.*, deletion, insertion, and substitution.

By way of example, preferred PYY analog polypeptides may include N-terminal deletions in combination with one or more amino acid substitutions. For instance, preferred PYY analog polypeptides include PYY (3-36) with the one or more of the following amino acid substitutions: Ala^3, Leu^3, Pro^3, Ala^4, Gly^4, d-Ala^4, homoLys^4, Glu^4, Ala^5, Ala^6, Val^6, d-Ala^7, Tyr^7, His^7, Ala^8, Ala^9, Ala^{10}, Ala^{11}, d-Ala^{12}, Ala^{13}, homoSer^{13}, Ala^{14}, Ala^{15}, Gln^{15}, Ala^{16}, Ala^{17}, Met^{17}, Ala^{18}, Ser^{18}, nor-Val^{18}, Ala^{19}, N-Me-Ala^{19}, Lys^{19}, homoArg^{19}, Ala^{20}, Ala^{21}, d-Ala^{22}, Ala^{23}, Ala^{24}, Ala^{25}, Lys^{25}, homoArg^{25}, Ala^{26}, Ala^{27}, Ala^{28}, Ala^{29}, Ala^{30}, Ala^{31}, Ala^{32}, Ala^{33}, Lys^{33}, Ala^{34}, Ala^{35}, Ala^{36}, His^{36}, Tip^{36}, N-Me-Tyr^{36}, and Phe^{36}. Preferably, the PYY analog polypeptide includes one, two, or three amino acid substitutions. Certain preferred PYY analog polypeptides comprising deletions in combination with amino acid insertions. *(see,* e.g., SEQ ID NOS: 89-174)

Preferred PYY analog polypeptides include the polypeptides of the Formula (V) (SEQ ID NO: 350):

Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaa_9 Xaa_{10} $Xaa\pi$ Xaa_{12}
Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr Xaa_{21} Xaa_{22}
Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_3oXaa_{31} Thr
Arg Gln Arg Xaa_{36}

wherein:

Xaa_3 is He, Ala, Pro, Ser, Thr, or NorVal;
Xaa_4 is Lys, Ala, Gly_5 Glu, Asp, d-Ala, homoLys, or homoArg;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaa_9 is Gly, Ala, Ser, sarcosine, Pro, or Aib;
Xaa_{10} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
$Xaa\pi$ is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, or homoSer;
Xaa_{14} is Pro, Ala, homoPro, hydroxyPro, Aib, or Gly;
Xaa_{15} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa_{16} is Glu, Ala, Asp, Asn, or Gln;

Xaa_{17} is Leu, Ala, Met, Trp, He, Val, or NorVal;
Xaa_{18} is Asn, Asp, Ala, Glu, Gln, Ser or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
$Xaa_{2}g$ is Leu or Ala;
Xaa_{30} is Leu, Ala, NorVal, or He;
Xaa_{31} is Ala or Val; and
Xaa_{36} is Tyr, N(Me)Tyr, His, or Trp;

with the proviso that said polypeptide is not a native PPF polypeptide.

As will be recognized by one of skill in the art, the polypeptides of Formula V may be in the free acid form, or may be C-terminally amidated.

Other preferred PYY analog polypeptides include SEQ ID NOs: 31-87, 93, 95, 96, 110, 114-116, 118, 120, 124-129, 131-132, 137-141, 146-156, 158, 160-164, 167-168, 170-171, 174-217, 221-222, 225, 228-229, 231-239, 242-245, 247-249, 251, 255-258, 260, 264, 266-286, and 288-347.

Also included within the scope of the invention are PYY analog polypeptides of Formulas II to V, wherein the indicated amino acid residue is chemical modified or derivitized (e.g., through faty acid derivitization, PEGylation, amidation, glycolization, etc.). Also contemplated within the scope of the invention are D-amino acid residues of the indicated amino acids.

In another embodiment, preferred PYY analog polypeptides include the polypeptides of Formulas II to V with internal deletions, particularly in areas not corresponding to the C-terminal tail PPF motif, as described herein.

Preferred PYY analog polypeptides comprising substitutions of unnatural amino acids include PYY(3-36), wherein amino acids at positions x and x+1 are substituted with β-turn mimetics selected from the group consisting of mimic A and mimic B, wherein x is selected from positions 8 to 14 *(see, e.g.,* SEQ ID NOS: 211-217 and 231-237).

Preferred derivatives of the PYY analog polypeptides of the invention include polymer-conjugated PYY analog polypeptides, wherein the PYY analog polypeptide includes any of the above-described insertions, deletions, substitutions, or combinations thereof, and the polymer molecule is conjugated at a lysine residue.

Other preferred derivatives of PYY analog polypeptides include PYY, PYY(3-36) or PYY(4-36) with the following substitutions and alterations: [Lys^{4}-fatty acid chain]PYY(3-36); [Lys^{4}-fatty acid chain]PYY(4-36); [Ala^{2}Lys^{19}-fatty acid chain]PYY(3-36); [Ile^{3}-fatty acid chain]PYY(3-36); [Ser^{13}-OAc] PYY(3-36) (OAc is O-Acylation with fatty acids or acetyl groups); [Ser^{23}-OAc]PYY(3-36); [Ile^{2}-Octanoyl chain]PYY(3-36); [Lys^{19}-Octanoyl chain]PYY(3-36); and [Lys^{19}-Stearyl chain]PYY(3-36).(>ee *e.g.,* SEQ ID NOS: 185-208).

Further examples of the PYY analog polypeptides of the present invention are provided in the Sequence Listing and discussed in the Examples section below.

PPF Chimeric Polypeptides

In yet another aspect of the invention, the PPF polypeptides of the invention include PPF chimeric polypeptides comprising a fragment of a PP, PYY or NPY polypeptide covalently linked to at least one additional fragment of a second PP, PYY or NPY polypeptide, wherein each PP, PYY or NPY fragment includes a PPF motif. Alternatively, the PPF chimeric polypeptides of the invention may comprise a fragment of a PP family polypeptide linked to one, two, three, or four polypeptides segments, wherein at least one of the linked polypeptide segments is a fragment of a second PP family polypeptide. In certain embodiments, PPF polypeptides do not include an N-terminal PP fragment with a C-terminal NPY fragment. PPF chimeric polypeptides of the invention will exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). In a preferred embodiment, such PPF chimeric polypeptides of the invention may exhibit at least 60%, 65%, 70%, 80%, or 90% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). Such PPF chimeric polypeptides of the invention may also exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native PP. In yet another embodiment, such PPF chimeric polypeptides of the invention may exhibit at least 50%, 60%, 65%, 70%, 80%, or 90% sequence identity to a native NPY. Further, the PPF chimeric polypeptides of the invention will preferably include at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif.

Again, the PPF polypeptides of the present invention will generally retain, at least in part, a biological activity of native human PP, PPY, or NPY. In a preferred

embodiment, the PPF chimeric polypeptides of the present invention will exhibit biological activity in the treatment and prevention of metabolic conditions and disorders.

The polypeptide fragments may be covalently linked together in any manner known in the art, including but not limited to direct amide bonds or chemical linker groups. Chemical linker groups may include peptide mimetics which induce or stabilize polypeptide conformation. Preferred PPF chimeric polypeptides of the invention include PYY-PP, PYY-NPY, PP-PYY, PP-NPY, NPY-PP, or NPY-PYY chimeras.

The PPF chimeric polypeptides of the invention may be at least 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 amino acids in length. Further, in a preferred embodiment, the PYY analog polypeptides of the invention include only natural L amino acid residues and/or modified natural L amino acid residues. Alternatively, in a preferred embodiment, the PYY analog polypeptides of the invention do not include unnatural amino acid residues.

Further, as mentioned above, the PPF chimeric polypeptides of the invention preferably do not include: hPP(l-7)-pNPY, hPP(l-17)-pNPY, hPP(19-23)-pNPY, hPP(19-23)-Pro^{34}pNPY, hPP(19-23)-His^{34}pNPY, rPP(19-23)-pNPY, rPP(19-23)-Pro^{34}pNPY, rPP(19-23)-His^{34}pNPY, hPP(l-17)-His^{34}pNPY, pNPY(l-7)-hPP, pNPY(l-7, 19-23)-hPP, cPP(l-7)-pNPY(19-23)-hPP, cPP(1-7)-NPY(19-23)-His^{34}hPP, hPP(l-17)-His^{34}pNPY, hPP(19-23)-pNPY, hPP(19-23)-Pro^{34}pNPY, pNPY(l-7)-hPP, pNPY(19-23)-hPP, pNPY(19-23)-Gln^{34}hPP, pNPY(19-23)-His^{34}hPP, $_{p}$NPY(19-23)-Phe^{6}Gln^{34}hPP, pNPY(19-23)-Phe^{6}His^{34}hPP, pNPY(l-7,19-23)-hPP, pNPY(l-7,19-23)-Gln^{34}hPP, cPP(20-23)-Pro^{34}-pNPY, cPP(21-23)-Pro^{34}-pNPY, cPP(22-23)-Pro^{34}-pNPY, cPP(l-7)-Pro^{34}-pNPY, cPP(20-23)-Pro^{34}-pNPY, cPP(l-7,20-23)-Pro^{34}-pNPY, cPP(l-7)-pNPY(19-23)-hPP, cPP(l-7)-pNPY(19-23)-His^{34}hPP, cPP(l-7)-gPP(19-23)-hPP, cPP(l-7)-pNPY(19-23)-Ala^{31}Aib^{32}Gln^{34}-hPP, cPP(l-7)-pNPY(19-23)-Ala^{31}Aib^{32}His^{34}-hPP hPP(l-7)-Ala^{31}AIb^{32}-pNPY$_{3}$ hPP(l-17)-AIa^{31}Aib^{3}^pNPY, pNPY(1-7)-Ala^{31}AIb^{32}GIn^{34}-hPP, or pNPY(l-7, 19-23)-Ala^{31}Aib^{32}Gln^{34}-hPP.

In a preferred embodiment, the PPF chimeric polypeptides of the invention may comprise fragments of PP family analog polypeptides. For instance, the PPF chimeric

polypeptides may comprise PPY analog polypeptides described herein, as well as PP analog polypeptides, and NPY analog polypeptides.

Preferred PYY analog polypeptide are those having a potency in one of the assays described herein (preferably food intake, gastric emptying, pancreatic secretion, or weight reduction assays) which is equal to or greater than the potency of NPY, PYY, or PYY(3-36) in that same assay. Alternatively, preferred PYY analog polypeptides of the invention may exhibit improved ease of manufacture, stability, and/or ease of formulation, as compared to PP, NPY, PYY, or PYY(3-36).

Preferably, the PPF chimeric polypeptides of the invention retain at least about 25%, preferably about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% percent of the biological activity of native human PYY with regard to the reduction of nutrient availability, the reduction of food intake, the effect of body weight gain, and/or the treatment and prevention of metabolic conditions and disorders. In another embodiment, the PPF chimeric polypeptides of the invention exhibit improved PYY agonist activity. Preferably, the PPF chimeric polypeptides of the invention exhibits at least about 110%, 125%, 130%, 140%, 150%, 200%, or more of the biological activity of native human PYY with regard to the reduction of nutrient availability the reduction of food intake, the effect of body weight gain, and/or the treatment and prevention of metabolic conditions and disorders.

More particularly, in one aspect, the PPF chimeric polypeptides preferably comprise a fragment of PP linked to a fragment of PYY. In one embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of PP or a PP analog polypeptide linked at its C-terminal end to a C-terminal fragment of PYY or a PYY analog polypeptide. In another embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of PYY, PYY(3-36), or a PYY analog polypeptide linked at its C-terminal end to a C-terminal fragment of PP or a PP analog polypeptide.

In another aspect, the PPF chimeric polypeptides preferably comprise a fragment of PYY linked to a fragment of NPY. In one embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of PYY, PYY(3-36), or a PYY analog polypeptide linked at its C-terminal end to a C-terminal fragment of

NPY or a NPY analog polypeptide. In another embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of NPY or a NPY analog polypeptide linked at its C-terminal end to a C-terminal fragment of PYY or a PYY analog polypeptide.

In yet another aspect, the PPF chimeric polypeptides preferably comprise a fragment of PP linked to a fragment of NPY. In one embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of PP or a PP analog polypeptide linked at its C-terminal end to a C-terminal fragment of NPY or a NPY analog polypeptide. In another embodiment, the PPF chimeric polypeptides of the invention comprise an N-terminal fragment of NPY or a NPY analog polypeptide linked at its C-terminal end to a C-terminal fragment of PP or a PP analog polypeptide.

A fragment of PP, a PP analog polypeptide, PYY, PYY(3-36)$_5$ a PYY analog polypeptide, NPY, or an NPY analog polypeptide is preferably a fragment comprising anywhere from 4 to 20 amino acid residues of the PP, PP analog polypeptide, PYY, PYY(3-36), PYY analog polypeptide, NPY, or NPY analog polypeptide. In a preferred embodiment, the length of fragment is selected so as to obtain a final PPF chimeric polypeptide of at least 34 amino acids in length.

The PPF chimeric polypeptides of the present invention may also comprise further modifications including, but are not limited to, substitution, deletion, and insertion to the amino acid sequence of such PPF chimeric polypeptides and any combination thereof. In a preferred aspect, the PPF chimeric polypeptides of the invention include one or more modifications of a "non-essential" amino acid residue. In the context of the invention, a "non-essential" amino acid residue is a residue that can be altered, *i.e.*, deleted or substituted, in the native human amino acid sequence of the fragment, *e.g.*, the PP family polypeptide fragment, without abolishing or substantially reducing the PYY agonist activity of the PPF chimeric polypeptide.

The present invention also relates to derivatives of the PPF chimeric polypeptides. Such derivatives include PPF chimeric polypeptides conjugated to one or more water soluble polymer molecules, such as polyethylene glycol ("PEG") or fatty acid chains of various lengths *(e.g.*, stearyl, palmitoyl, octanoyl, oleoyl etc.), or by the addition of

polyamino acids, such as poly-his, poly-arg, poly-lys, and poly-ala. Modifications to the PPF chimeric polypeptides can also include small molecule substituents, such as short alkyls and constrained alkyls *(e.g.,* branched, cyclic, fused, adamantyl), and aromatic groups. The water soluble polymer molecules will preferably have a molecular weight ranging from about 500 to about 20,000 Daltons.

Such polymer-conjugations and small molecule substituent modifications may occur singularly at the N- or C-terminus or at the side chains of amino acid residues within the sequence of the PPF chimeric polypeptides. Alternatively, there may be multiple sites of derivatization along the PPF chimeric polypeptide. Substitution of one or more amino acids with lysine, aspartic acid, glutamic acid, or cysteine may provide additional sites for derivatization. *See, e.g.,* U.S. Patent Nos. 5,824,784 and 5,824,778. Preferably, the PPF chimeric polypeptides may be conjugated to one, two, or three polymer molecules.

The water soluble polymer molecules are preferably lined to an amino, carboxyl, or thiol group, and may be linked by N or C terminus, or at the side chains of lysine, aspartic acid, glutamic acid, or cysteine. Alternatively, the water soluble polymer molecules may be linked with diamine and dicarboxylic groups. In a preferred embodiment, the PPF chimeric polypeptides of the invention are conjugated to one, two, or three PEG molecules through an epsilon amino group on a lysine amino acid.

PPF chimeric polypeptide derivatives of the invention also include PPF chimeric polypeptides with chemical alterations to one or more amino acid residues. Such chemical alterations include amidation, glycosylation, acylation, sulfation, phosphorylation, acetylation, and cyclization. The chemical alterations may occur singularly at the N- or C-terminus or at the side chains of amino acid residues within the sequence of the PPF chimeric polypeptides. In one embodiment, the C-terminus of these peptides may have a free -OH or $-NH_2$ group. In another embodiment, the N-terminal end may be capped with an isobutyloxycarbonyl group, an isopropyloxycarbonyl group, an n-butyloxycarbonyl group, an ethoxycarbonyl group, an isocaproyl group (isocap), an octanyl group, an octyl glycine group (G(Oct)), or an 8-aminooctanic acid group. In a preferred embodiment, cyclization can be through the formation of disulfide bridges. Alternatively, there may be multiple sites of chemical alteration along the PYY analog polypeptide.

In a preferred aspect, the PPF chimeric polypeptides include those having an amino acid sequence of SEQ ID NOs. 238-347.

Examples of the PPF chimeric polypeptides of the present invention are provided in the Sequence Listing and further discussed in the Examples section below.

Use of PPF Polypeptides in the Treatment or Prevention of Metabolic Conditions or Disorders

It has been generally accepted that endogenous NPY (reviewed in Schwartz et al., Nature 404: 661-71 (2000)) and PYY (Morley et al., Brain Res. 341: 200-3 (1985)), via their receptors, increase feeding behavior. Methods directed at therapies for obesity have invariably attempted to antagonize Y receptors, while claims for treating anorexia have been directed at agonists of this ligand family. However, as described and claimed in the commonly-owned pending U.S. Patent Application No. 20020141985, it has been surprisingly discovered that peripheral administration of PYY analog polypeptides has a potent effect to reduce nutrient availability (see also Batterham *et al,* Nature 418: 650-4, 2002; WO 03/026591; and WO 03/057235), rather than increase it as suggested by reports in the patent and scientific literature *(see, e.g.,* U.S. Patent Nos. 5,912,227 and 6,315,203 which disclose the use of PYY receptor agonists to increase weight gain). The spectrum of actions of inhibition of food intake, slowing of gastric emptying, inhibition of gastric acid secretion, and inhibition of pancreatic enzyme secretion, are useful to exert clinical benefit in metabolic diseases such as type 1, type 2, or gestational diabetes mellitus, obesity and other manifestations of insulin-resistance syndrome (Syndrome X), and in any other use for reducing nutrient availability.

As such, in another aspect of the invention, methods for treating or preventing obesity are provided, wherein the method comprises administering a therapeutically or prophylactically effective amount of a PPF polypeptide to a subject in need thereof. In a preferred embodiment, the subject is an obese or overweight subject. While "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese." Subjects who are insulin resistant, glucose intolerant, or have any form of diabetes mellitus *(e.g.,* type 1, 2 or gestational diabetes) can benefit from this method.

In other aspects of the invention, methods of reducing food intake, reducing nutrient availability, causing weight loss, affecting body composition, and altering body energy content or increasing energy expenditure, treating diabetes mellitus, and improving lipid profile (including reducing LDL cholesterol and triglyceride levels and/or changing HDL cholesterol levels) are provided, wherein the methods comprise administering to a subject an effective amount of a PPF polypeptide of the invention. In a preferred embodiment, the methods of the invention are used to treat or prevent conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of a PPF polypeptide of the invention. Such conditions and disorders include, but are not limited to, hypertension, dyslipidemia, cardiovascular disease, eating disorders, insulin-resistance, obesity, and diabetes mellitus of any kind.

Without intending to be limited by theory, it is believed that the effects of peripherally-administered PPF polypeptides of the present invention in the reduction of food intake, in the delay of gastric emptying, in the reduction of nutrient availability, and in the causation of weight loss are determined by interactions with one or more unique receptor classes in, or similar to, those in the PP family. More particularly, it appears that a receptor or receptors similar to the PYY-preferring (or Y7) receptors are involved.

Additional assays useful to the invention include those that can determine the effect of PPF compounds on body composition. An exemplary assay can be one that involves utilization of a diet-induced obese (DIO) mouse model for metabolic disease. Prior to the treatment period, male C57BL/6J mice can be fed a high-fat diet (#D12331, 58% of calories from fat; Research Diets, Inc.) for 6 weeks beginning at 4 weeks of age. During the study, the mice can continue to eat their high-fat diet. Water can be provided *ad libitum* throughout the study. One group of similarly-aged non-obese mice can be fed a low-fat diet (#D12329, 11% of calories from fat) for purposes of comparing metabolic parameters to DIO groups.

DIO mice can be implanted with subcutaneous (SC) intrascapular osmotic pumps to deliver either vehicle (50% dimethylsulfoxide [DMSO] in water) n=20 or a compound

of the invention n=12. The pumps of the latter group can be set to deliver any amount, e.g., 1000 µg/kg/d of a compound of the invention for 7 days.

Body weights and food intake can be measured over regular intervals throughout the study periods. Respiratory quotient (RQ, defined as CO_2 production ÷ O_2 consumption) and metabolic rate can be determined using whole-animal indirect calorimetry (Oxymax, Columbus Instruments, Columbus, OH). The mice can be euthanized by isoflurane overdose, and an index of adiposity (bilateral epididymal fat pad weight) measured. Moreover, prior to determination of epididymal weight, body composition (lean mass, fat mass) for each mouse can be analyzed using a Dual Energy X-ray Absorptiometry (DEXA) instrument per manufacturer's instructions (Lunar Piximus, GE Imaging System). In the methods of the invention, preferred PPF polypeptide of the invention are those having a potency in one of the assays described herein (preferably food intake, gastric emptying, pancreatic secretion, weight reduction or body composition assays) which is greater than the potency of PP, NPY, PYY, or PYY(3-36) in that same assay.

In addition to the amelioration of hypertension in subjects in need thereof as a result of reduced food intake, weight loss, or treating obesity, compounds of the invention may be used to treat hypotension as described in Example 4.

Compounds of the invention may also be useful for potentiating, inducing, enhancing or restoring glucose responsivity in pancreatic islets or cells. These actions may be useful for treating or preventing conditions associated with metabolic disorders such as those described above and in U.S. patent application no. US20040228846. Assays for determining such activity are known in the art. For example, in published U.S. patent application no. US20040228846 (incorporated by reference in its entirety), assays are described for islet isolation and culture as well as determining fetal islet maturation. In the examples of patent application US20040228846, intestine-derived hormone peptides including pancreatic polypeptide (PP), neuropeptide Y (NPY), neuropeptide K (NPK), PYY, secretin, glucagon-like peptide-1 (GLP-I) and bombesin were purchased from Sigma. Collagenase type XI was obtained from Sigma. RPMI 1640 culture medium and fetal bovine serum were obtained from Gibco. A radioimmunoassay kit containing anti-insulin antibody ([^{125}I]-RIA kit) was purchased from Linco, St Louis.

Post-partem rat islets were obtained from P-02 year old rats. Adult rat islets were obtained from 6-8 week old rats. Fetal rat islets were obtained as follows. Pregnant female rats were sacrificed on pregnancy day e21. Fetuses were removed from the uterus. 10-14 pancreata were dissected from each litter and washed twice in Hanks buffer. The pancreas were pooled, suspended in 6 ml 1 mg/ml collagenase (Type XI, Sigma) and incubated at 37° C for 8-10 minutes with constant shaking. The digestion was stopped by adding 10 volumes of ice-cold Hanks buffer followed by three washes with Hanks buffer. The islets were then purified by Ficoll gradient and cultured in 10% fetal bovine serum (FBS)/RPMI medium with or without addition of 1 μM IBMX. At the end of five days, 20 islets were hand picked into each tube and assayed for static insulin release. Generally, islets were first washed with KRP buffer and then incubated with 1 ml of KRP buffer containing 3 mM (low) glucose for 30 minutes at 37° C. with constant shaking. After collecting the supernatant, the islets were then incubated with 17 mM (high) glucose for one hour at 37° C. The insulin released from low or high glucose stimulation were assayed by radioimmunoassay (RIA) using the [^{125}I]-RIA kit. E21 fetal islets were cultured for 5 days in the presence of 200 ng/ml PYY, PP, CCK, NPK, NPY, Secretin, GLP-I or Bombesin.

An exemplary *in vivo* assays is also provided using the Zucker Diabetic Fatty (ZDF) male rat, an inbred (>F30 Generations) rat model that spontaneously expresses diabetes in all fa/fa males fed a standard rodent diet Purina 5008. In ZDF fa-fa males, hyperglycemia begins to develop at about seven weeks of age and glucose levels (fed) typically reach 500 mg/DL by 10 to 11 weeks of age. Insulin levels (fed) are high during the development of diabetes. However, by 19 weeks of age insulin drops to about the level of lean control litter mates. Triglyceride and cholesterol levels of obese rats are normally higher than those of leans. In the assay, three groups of 7-week old ZDF rats, with 6 rats per group, received the infusion treatment by ALZA pump for 14 days: 1) vehicle control, 2) and 3), PYY with two different doses, 100 pmol/kg/hr and 500 pmol/kg/hr respectively. Four measurements were taken before the infusion and after the infusion at day 7 and day 14: 1) plasma glucose level, 2) plasma insulin level, and 3) plasma triglycerides (TG) level, as well as oral glucose tolerance (OGTT) test. Accordingly, these assays can be used with compounds of the invention to test for desired activity.

Other uses contemplated for the PPF polypeptides include methods for reducing aluminum (Al) concentrations in the central nervous system (see U.S. Pat. 6,734,166, incorporated by reference in its entirety) for treating, preventing, or delay the onset of Alzheimer's disease. Assays for determining effects on Al are known in the art an dean be found in US Pat 6,734,166 using diploid and Ts mice. These mice were individually housed in Nalgene® brand metabolism or polypropylene cages and given three days to adjust to the cages before experimentation. Mice had free access to food (LabDiet® NIH Rat and Moust/Auto 6F5K52, St. Louis, Mo.) and water during the experiment except for the 16 hours prior to euthanasia when no food was provided. Mice were given daily subcutaneous injections of either active compound or saline. Mice were sacrificed at the end of day 13 for one experiment and day 3 for another, and samples were collected. Mice brain samples were weighted in clean teflon liners and prepared for analysis by microwave digestion in low trace element grade nitric acid. Sample were then analyzed for Al content using Inductively Coupled Plasma Mass Spectrometry (Nuttall et al., Annals of Clinical and Laboratory Science 25, 3, 264-271 (1995)). All tissue handling during analysis took place in a clean room environment utilizing HEPA air filtration systems to minimize background contamination.

The compounds of the invention exhibit a broad range of biological activities, some related to their antisecretory and antimotility properties. The compounds may suppress gastrointestinal secretions by direct interaction with epithelial cells or, perhaps, by inhibiting secretion of hormones or neurotransmitters which stimulate intestinal secretion. Anti-secretory properties include inhibition of gastric and/or pancreatic secretions and can be useful in the treatment or prevention of diseases and disorders including gastritis, pancreatitis, Barrett's esophagus, and Gastroesophageal Reflux Disease.

Compounds of the invention are useful in the treatment of any number of gastrointestinal disorders (see *e.g.,* Harrison's Principles of Internal Medicine, McGraw-Hill Inco, New York, 12th Ed.) that are associated with excess intestinal electrolyte and water secretion as well as decreased absorption, *e.g.,* infectious diarrhea, inflammatory diarrhea, short bowel syndrome, or the diarrhea which typically occurs following surgical procedures, *e.g.,* ileostomy. Examples of

infectious diarrhea include, without limitation, acute viral diarrhea, acute bacterial diarrhea *(e.g.,* salmonella, Campylobacter, and Clostridium or due to protozoal infections), or traveller's diarrhea *(e.g.,* Norwalk virus or rotavirus). Examples of inflammatory diarrhea include, without limitation, malabsorption syndrome, tropical sprue, chronic pancreatitis, Crohn's disease, diarrhea, and irritable bowel syndrome. It has also been discovered that the peptides of the invention can be used to treat an emergency or life-threatening situation involving a gastrointestinal disorder, e.g., after surgery or due to cholera.

Compounds of the invention may also be useful for treating or preventing intestinal damage as opposed to merely treating the symptoms associated with the intestinal damage (for example, diarrhea). Such damage to the intestine may be, or a result of, ulcerative colitis, inflammatory bowel disease, bowel atrophy, loss bowel mucosa, and/or loss of bowel mucosal function (see WO 03/105763, incorporated herein by reference in its entirety). Assays for such activity, as described in WO 03/105763, include 11 week old male HSD rats, ranging 250- 300 grams housed in a 12:12 lightdark cycle, and allowed ad libitum access to a standard rodent diet (Teklad LM 485, Madison, WI) and water. The animals were fasted for 24 hours before the experiment. A simple and reproducible rat model of chronic colonic inflammation has been previously described by Morris GP, et al., "Hapten- induced model of chronic inflammation and ulceration in the rat colon." Gastroenterology. 1989; 96:795-803. It exhibits a relatively long duration of inflammation and ulceration, affording an opportunity to study the pathophysiology of colonic inflammatory disease in a specifically controlled fashion, and to evaluate new treatments potentially applicable to inflammatory bowel disease in humans.

Rats were anesthetized with 3% isofluorane and placed on a regulated heating pad set at 37°C. A gavage needle was inserted rectally into the colon 7 cm. The hapten trinitrobenzenesulfonic acid (TNBS) dissolved in 50% ethanol (v/v) was delivered into the lumen of the colon through the gavage needle at a dose of 30 mg/kg, in a total volume of 0 0.4-0.6 mL, as described in Mazelin, et al., "Protective role of vagal afferents in experimentally-induced colitis in rats." Juton Nerv Syst. 1998;73:38 45. Control groups received saline solution (NaCl 0.9%) intracolonically.

Four days after induction of colitis, the colon was resected from anesthetized rats, which were then euthanized by decapitation. Weights of excised colon and spleen were measured, and the colons photographed for scoring of gross morphologic damage. Inflammation was defined as regions of hyperemia and bowel wall thickening.

Compounds of the invention may also be used to treat or prevent pancreatic tumors (*e.g.*, inhibit the proliferation of pancreatic tumors). Methods of the invention include reducing the proliferation of tumor cells. The types of benign pancreatic tumor cells which may be treated in accordance with the present invention include serous cyst adenomas, microcystic tumors, and solid-cystic tumors. The method is also effective in reducing the proliferation of malignant pancreatic tumor cells such as carcinomas arising from the ducts, acini, or islets of the pancreas. U.S. Pat. 5,574,010 (incorporated by reference in its entirety) provides exemplary assays for testing anti¬proliferative properties. For example, the '010 patent provides that PANC-I and MiaPaCa-2 are two human pancreatic adenocarcinoma cancer cell lines which are available commercially from suppliers such as American Type Culture Collection, ATCC (Rockville, Md.). The two tumor cells were grown in RPMI- 1640 culture media supplemented with 10% fetal bovine serum, 29.2 mg/L of glutamine, 25 .mu.g gentamicin, 5 ml penicillin, streptomycin, and fungizone solution (JRH Biosciences, Lenexa, Kans.) at 37 degrees Celcius in a NAPCO water jacketed 5 % CO.sub.2 incubator. All cell lines were detached with 0.25 % trypsin (Clonetics, San Diego, Calif.) once to twice a week when a confluent monolayer of tumor cells was achieved. Cells were pelleted for 7 minutes at 500 g in a refrigerated centrifuge at 4 degrees Celcius, and resuspended in trypsin free fortified RPMI 1640 culture media. Viable cells were counted on a hemocytometer slide with trypan blue.

Ten thousand, 20,000, 40,000 and 80,000 cells of each type were added to 96 well microculture plates (Costar, Cambridge, Mass.) in a total volume of 200 ul of culture media per well. Cells were allowed to adhere for 24 hours prior to addition of the PYY or test peptide. Fresh culture media was exchanged prior to addition of peptides. In vitro incubation of pancreatic tumor cells with either PYY or test compound was continued for 6 hours and 36 hours in length. PYY was added to cells at doses of 250 pmol, 25 pmol, and 2.5 pmol per well (N =14). Test compound was added to cells

cultures at doses of 400 pmol, 40 pmol, and 4 pmol per well. Control wells received 2 ul of 0.9% saline to mimic the volume and physical disturbance upon adhered tumor cells. Each 96 well plate contained 18 control wells to allow for comparison within each plate during experimentation. Ninety-six *(96)* well plates were repeated 6 times with varying concentrations of PYY and test compound in both the PANC-I and MiaPaCa-2 cells.

At the end of the incubation period, 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium Bromide, MTT tetrazolium bromide (Sigma, St. Louis, Mo.) was added to fresh culture media at 0.5 mg/ml. Culture media was exchanged and tumor cells were incubated for 4 hours with MTT tetrazolium bromide at 37 degrees Celcius. At the end of incubation, culture media was aspirated. Formazon crystal precipitates were dissolved in 200 ul of dimethyl sulfoxide (Sigma, St. Louis, Mo.). Quantitation of solubilized formazon was performed by obtaining absorption readings at 500 nm wavelength on an ELISA reader (Molecular Devices, Menlo Park, Calif). The MTT assay measures mitochondrial NADH dependent dehydrogenase activity, and it has been among the most sensitive and reliable method to quantitative in vitro chemotherapy responses of tumor cells. (Alley, M. C, Scudiero, D. A., Monk, A., Hursey, M. L., Dzerwinski, M. J., Fine, D. *L.*, Abbott, B. J., Mayo, J. G., Shoemaker, R. H. and Boyd, M. R., Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay Cancer Res., 48:589-601, 1988; Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. and Mitchell, J. B., Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. Cancer Res., 47:936-942, 1987; McHale$_3$A. P., McHale, L., Use of a tetrazolium based colorimetric assay in assessing photoradiation therapy in vitro. Cancer Lett., 41:315-321, 1988; and Saxton, R. E., Huang, M. Z., Plante D., Fetterman, H. F., Lufkin, R. B., Soudant, J., Castro, D. J., Laser and daunomycin chemophototherapy of human carcinoma cells. J. Clin. Laser Med. and Surg., 10(5):33 1-336, 1992.) Analysis of absorption readings at 550 nm were analyzed by grouping wells of the same test conditions and verifying differences occurring between control and the various peptide concentration treatments by one-way ANOVA.

An exemplary in vivo assay is also provided. The human pancreatic ductal adenocarcinoma Mia Paca-2 was examined for in vivo growth inhibition by peptide YY and test compound. Seventy thousand to 100,000 human Mia PaCa-2 cells were orthotopically transplanted into 48 male athymic mice. After one week, the animals were treated with either PYY or test compound at 200 pmol/kg/hr via mini-osmotic pumps for four weeks. The paired cultures received saline. At sacrifice, both tumor size and mass were measured. Control mice had significant human cancer growth within the pancreas as evidenced by histologic sections. At 9 weeks, ninety percent (90%) of control mice had substantial metastatic disease. Tumor mass was decreased by 60.5 % in test treated mice and 27% in PYY treated mice.

For all indications, in preferred embodiments, the PPF polypeptide of the invention is administered peripherally at a dose of about 0.5 μg to about 5 mg per day in single or divided doses or controlled continual release, or at about 0.01 μg/kg to about 500 μg/kg per dose, more preferably about 0.05 μg/kg to about 250 μg/kg, most preferably below about 50 μg/kg. Dosages in these ranges will vary with the potency of each analog or derivative, of course, and may be determined by one of skill in the art.

In the methods of the present invention, PPF polypeptides of the invention may be administered separately or together with one or more other compounds and compositions that exhibit a long term or short-term action to reduce nutrient availability, including, but not limited to other compounds and compositions that comprise an amylin or amylin analog agonist, salmon calcitonin, a cholecystokinin (CCK) or CCK agonist, a leptin (OB protein) or leptin agonist, an exendin or exendin analog agonist, or a GLP-I or GLP-I analog agonist. Suitable amylin agonists include, for example, [^{25,28,29}Pro-] human amylin (also known as "pramlintide," and described in U.S. Pat. Nos. 5,686,51 1 and 5,998,367). The CCK used is preferably CCK octopeptide (CCK-8). Leptin is discussed in, for example, (Pelleymounter et al., Science 269: 540-3 (1995); Halaas et al., Science 269: 543-6 (1995); Campfield et al., Science 269: 546-9 (1995)). Suitable exendins include exendin-3 and exendin-4, and exendin agonist compounds include, for example, those described in PCT Publications WO 99/07404, WO 99/25727, and WO 99/25728.

Polypeptide Production and Purification

The PPF polypeptides described herein may be prepared using standard recombinant techniques or chemical peptide synthesis techniques known in the art, *e.g.*, using an automated or semi-automated peptide synthesizer, or both.

The PPF polypeptides of the invention can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. *See, e.g.*, Stewart and Young, Solid Phase Peptide Synthesis, 2d. *ed*, Pierce Chemical Co. (1984); Tam *et al*, J. Am. Chem. Soc. 105: 6442 (1983); Merrifield, Science 232: 341-7 (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds., Academic Press, New York, 1-284 (1979). Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer *(e.g.*, Model 430A, Applied Biosystems Inc., Foster City, California) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry *(see*, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, California) with capping. Peptides may also be assembled using an Advanced ChemTech Synthesizer (Model MPS 350, Louisville, Kentucky). Peptides may be purified by RP-HPLC (preparative and analytical) using, e.g., a Waters Delta Prep 3000 system and a C4, C8, or C18 preparative column (10 μ, 2.2x25 cm; Vydac, Hesperia, California). The active protein can be readily synthesized and then screened in screening assays designed to identify reactive peptides.

The PPF polypeptides of the present invention may alternatively be produced by recombinant techniques well known in the art. *See, e.g.*, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d *ed*, Cold Spring Harbor (1989). These PYY analog polypeptides produced by recombinant technologies may be expressed from a polynucleotide. One skilled in the art will appreciate that the polynucleotides, including DNA and RNA, that encode such encoded PYY analog polypeptides may be obtained from the wild-type PYY cDNA, taking into consideration the degeneracy of codon usage. These polynucleotide sequences may incorporate codons facilitating transcription and translation of mRNA in microbial hosts. Such manufacturing sequences may readily be constructed according to the methods well known in the art.

See, e.g., WO 83/04053. The polynucleotides above may also optionally encode an N-terminal methionyl residue. Non-peptide compounds useful in the present invention may be prepared by art-known methods. For example, phosphate-containing amino acids and peptides containing such amino acids may be prepared using methods known in the art. *See, e.g.,* Bartlett and Landen, Bioorg. Chem. 14: 356-77 (1986).

A variety of expression vector/host systems may be utilized to contain and express a PPF polypeptide coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors *(e.g.,* baculovirus); plant cell systems transfected with virus expression vectors *(e.g.,* cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors *(e.g.,* Ti or pBR322 plasmid); or animal cell systems. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), WI 38, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the protein are described herein below.

As such, polynucleotide sequences provided by the invention are useful in generating new and useful viral and plasmid DNA vectors, new and useful transformed and transfected procaryotic and eucaryotic host cells (including bacterial, yeast, and mammalian cells grown in culture), and new and useful methods for cultured growth of such host cells capable of expression of the present PPF polypeptides. The polynucleotide sequences encoding PPF polypeptides herein may be useful for gene therapy in instances where underproduction of PP, PYY, or NPY would be alleviated, or the need for increased levels of such would be met.

The present invention also provides for processes for recombinant DNA production of the present PPF polypeptides. Provided is a process for producing the PPF polypeptides from a host cell containing nucleic acids encoding such PPF polypeptides comprising: (a) culturing said host cell containing polynucleotides

encoding such PPF polypeptides under conditions facilitating the expression of such DNA molecule; and (b) obtaining such PPF polypeptides.

Host cells may be prokaryotic or eukaryotic and include bacteria, mammalian cells (such as Chinese Hamster Ovary (CHO) cells, monkey cells, baby hamster kidney cells, cancer cells or other cells), yeast cells, and insect cells.

Mammalian host systems for the expression of the recombinant protein also are well known to those of skill in the art. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing, which cleaves a "prepro" form of the protein, may also be important for correct insertion, folding and/or function. Different host cells, such as CHO, HeLa, MDCK, 293, WI38, and the like, have specific cellular machinery and characteristic mechanisms for such post-translational activities, and may be chosen to ensure the correct modification and processing of the introduced foreign protein.

Alternatively, a yeast system may be employed to generate the PPF polypeptides of the present invention. The coding region of the PPF polypeptide cDNA is amplified by PCR. A DNA encoding the yeast pre-pro-alpha leader sequence is amplified from yeast genomic DNA in a PCR reaction using one primer containing nucleotides 1-20 of the alpha mating factor gene and another primer complementary to nucleotides 255-235 of this gene (Kurjan and Herskowitz, Cell, 30: 933-43 (1982)). The pre-pro-alpha leader coding sequence and PPF polypeptide coding sequence fragments are ligated into a plasmid containing the yeast alcohol dehydrogenase (ADH2) promoter, such that the promoter directs expression of a fusion protein consisting of the pre-pro-alpha factor fused to the mature PPF polypeptide. As taught by Rose and Broach, Meth. Enz. 185: 234-79, Goeddel ed., Academic Press, Inc., San Diego, California (1990), the vector further includes an ADH2 transcription terminator downstream of the cloning site, the yeast "2-micron" replication origin, the yeast leu-2d gene, the yeast REPl and REP2 genes, the E. coli β-lactamase gene, and an E. coli origin of replication. The β-lactamase and leu-2d genes provide for selection in bacteria and yeast, respectively. The leu-2d gene also facilitates increased copy number of the

plasmid in yeast to induce higher levels of expression. The REPl and REP2 genes encode proteins involved in regulation of the plasmid copy number.

The DNA construct described in the preceding paragraph is transformed into yeast cells using a known method, *e.g.*, lithium acetate treatment (Steams *et al,* Meth. Enz. 185: 280-97 (1990)). The ADH2 promoter is induced upon exhaustion of glucose in the growth media (Price *et al,* Gene 55: 287 (1987)). The pre-pro-alpha sequence effects secretion of the fusion protein from the cells. Concomitantly, the yeast KEX2 protein cleaves the pre-pro sequence from the mature PYY analog polypeptides (Bitter *et al,* Proc. Natl. Acad. Sci. USA 81: 5330-4 (1984)).

PPF polypeptides of the invention may also be recombinantly expressed in yeast using a commercially available expression system, *e.g.*, the Pichia Expression System (Invitrogen, San Diego, California), following the manufacturer's instructions. This system also relies on the pre-pro-alpha sequence to direct secretion, but transcription of the insert is driven by the alcohol oxidase (AOXl) promoter upon induction by methanol. The secreted PPF polypeptide is purified from the yeast growth medium by, *e.g.*, the methods used to purify PPF polypeptide from bacterial and mammalian cell supernatants.

Alternatively, the cDNA encoding PYY analog polypeptides may be cloned into the baculovirus expression vector pVL1393 (PharMingen, San Diego, California). This PPF polypeptides-containing vector is then used according to the manufacturer's directions (PharMingen) to infect Spodoptera frugiperda cells in sF9 protein-free media and to produce recombinant protein. The protein is purified and concentrated from the media using a heparin-Sepharose column (Pharmacia, Piscataway, New Jersey) and sequential molecular sizing columns (Amicon, Beverly, Massachusetts), and resuspended in PBS. SDS-PAGE analysis shows a single band and confirms the size of the protein, and Edman sequencing on a Proton 2090 Peptide Sequencer confirms its N-terminal sequence.

For example, the DNA sequence encoding the predicted mature PYY analog polypeptide may be cloned into a plasmid containing a desired promoter and, optionally, a leader sequence (*see, e.g.*, Better et al., Science 240: 1041-3 (1988)). The sequence of this construct may be confirmed by automated sequencing. The

plasmid is then transformed into E. coli, strain MC1061, using standard procedures employing CaC12 incubation and heat shock treatment of the bacteria (Sambrook *et al., supra).* The transformed bacteria are grown in LB medium supplemented with carbenicillin, and production of the expressed protein is induced by growth in a suitable medium. If present, the leader sequence will affect secretion of the mature PYY analog polypeptide and be cleaved during secretion. The secreted recombinant protein is purified from the bacterial culture media by the method described herein below.

Alternatively, the PPF polypeptides of the invention may be expressed in an insect system. Insect systems for protein expression are well known to those of skill in the art. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The PPF polypeptide coding sequence is cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of PYY analog polypeptide will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect S. frugiperda cells or Trichoplusia larvae in which PYY analog polypeptide is expressed (Smith *et al,* J. Virol. 46: 584 (1983); Engelhard *et al,* Proc. Natl. Acad. Sci. USA 91: 3224-7 (1994)).

In another example, the DNA sequence encoding the PPF polypeptide may be amplified by PCR and cloned into an appropriate vector, for example, pGEX-3X (Pharmacia, Piscataway, New Jersey). The pGEX vector is designed to produce a fusion protein comprising glutathione-S-transferase (GST), encoded by the vector, and a protein encoded by a DNA fragment inserted into the vector's cloning site. The primers for the PCR may be generated to include, for example, an appropriate cleavage site. The recombinant fusion protein may then be cleaved from the GST portion of the fusion protein. The pGEX-3X/PYY analog polypeptide construct is transformed into E. coli XL-I Blue cells (Stratagene, La Jolla, California), and individual transformants are isolated and grown at 37°C in LB medium (supplemented with carbenicillin) to an optical density at wavelength 600 nm of 0.4, followed by further incubation for 4 hours in the presence of 0.5 mM Isopropyl β-D-

Thiogalactopyranoside (Sigma Chemical Co., St. Louis, Missouri). Plasmid DNA from individual transformants is purified and partially sequenced using an automated sequencer to confirm the presence of the desired PPF polypeptide-encoding gene insert in the proper orientation.

The fusion protein, expected to be produced as an insoluble inclusion body in the bacteria, may be purified as follows. Cells are harvested by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 niM EDTA; and treated with 0.1 mg/mL lysozyme (Sigma Chemical Co.) for 15 min. at room temperature. The lysate is cleared by sonication, and cell debris is pelleted by centrifugation for 10 min. at 12,000xg. The fusion protein-containing pellet is resuspended in 50 mM Tris, pH 8, and 10 mM EDTA, layered over 50% glycerol, and centrifuged for 30 min. at 6000xg. The pellet is resuspended in standard phosphate buffered saline solution (PBS) free of Mg^{++} and Ca^{++}. The fusion protein is further purified by fractionating the resuspended pellet in a denaturing SDS polyacrylamide gel (Sambrook et al., supra). The gel is soaked in 0.4 M KCl to visualize the protein, which is excised and electroeluted in gel-running buffer lacking SDS. If the GST/PYY analog polypeptide fusion protein is produced in bacteria as a soluble protein, it may be purified using the GST Purification Module (Pharmacia Biotech).

The fusion protein may be subjected to digestion to cleave the GST from the mature PYY analog polypeptide. The digestion reaction (20-40 µg fusion protein, 20-30 units human thrombin (4000 U/mg (Sigma) in 0.5 mL PBS) is incubated 16-48 hrs. at room temperature and loaded on a denaturing SDS-PAGE gel to fractionate the reaction products. The gel is soaked in 0.4 M KCl to visualize the protein bands. The identity of the protein band corresponding to the expected molecular weight of the PYY analog polypeptide may be confirmed by partial amino acid sequence analysis using an automated sequencer (Applied Biosystems Model 473A, Foster City, California).

In a particularly preferred method of recombinant expression of the PPF polypeptides of the present invention, 293 cells may be co-transfected with plasmids containing the PYY analog polypeptide cDNA in the pCMV vector (5' CMV promoter, 3' HGH poly A sequence) and pSV2neo (containing the neo resistance gene) by the calcium phosphate method. Preferably, the vectors should be linearized with Seal prior to

transfection. Similarly, an alternative construct using a similar pCMV vector with the neo gene incorporated can be used. Stable cell lines are selected from single cell clones by limiting dilution in growth media containing 0.5 mg/mL G418 (neomycin-like antibiotic) for 10-14 days. Cell lines are screened for PYY analog polypeptide expression by ELISA or Western blot, and high-expressing cell lines are expanded for large scale growth.

It is preferable that the transformed cells are used for long-term, high-yield protein production and as such stable expression is desirable. Once such cells are transformed with vectors that contain selectable markers along with the desired expression cassette, the cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The selectable marker is designed to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell.

A number of selection systems may be used to recover the cells that have been transformed for recombinant protein production. Such selection systems include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, that confers resistance to methotrexate; gpt, that confers resistance to mycophenolic acid; neo, that confers resistance to the aminoglycoside, G418; also, that confers resistance to chlorsulfuron; and hygro, that confers resistance to hygromycin. Additional selectable genes that may be useful include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, β-glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

Many of the PPF polypeptides of the present invention may be produced using a combination of both automated peptide synthesis and recombinant techniques. For example, a PPF polypeptide of the present invention may contain a combination of modifications including deletion, substitution, and insertion by PEGylation. Such a

PPF polypeptide may be produced in stages. In the first stage, an intermediate PPF polypeptide containing the modifications of deletion, substitution, insertion, and any combination thereof, may be produced by recombinant techniques as described. Then after an optional purification step as described below, the intermediate PPF polypeptide is PEGylated through chemical modification with an appropriate PEGylating reagent *(e.g.,* from NeKtar Therapeutics, San Carlos, California) to yield the desired PPF polypeptide. One skilled in the art will appreciate that the above-described procedure may be generalized to apply to a PPF polypeptide containing a combination of modifications selected from deletion, substitution, insertion, derivation, and other means of modification well known in the art and contemplated by the present invention.

It may be desirable to purify the PPF polypeptides generated by the present invention. Peptide purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography, polyacrylamide gel electrophoresis, and isoelectric focusing. A particularly efficient method of purifying peptides is reverse phase HPLC, followed by characterization of purified product by liquid chromatography/mass spectrometry (LC/MS) and Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry. Additional confirmation of purity is obtained by determining amino acid analysis.

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded protein or peptide. The term "purified peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the peptide is purified to any degree relative to its naturally obtainable state. A purified peptide therefore also refers to a peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the peptides in the composition.

Various techniques suitable for use in peptide purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies, and the like; heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

There is no general requirement that the peptides always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different foπns of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed, utilizing an HPLC apparatus, will generally result in a greater "-fold" purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

One may optionally purify and isolate such PPF polypeptides from other components obtained in the process. Methods for purifying a polypeptide can be found in U.S. Patent No. 5,849,883. These documents describe specific exemplary methods for the isolation and purification of G-CSF compositions that may be useful in isolating and purifying the PPF polypeptides of the present invention. Given the disclosure of these patents, it is evident that one of skill in the art would be well aware of numerous

purification techniques that may be used to purify PPF polypeptides from a given source.

Also it is contemplated that a combination of anion exchange and immunoaffinity chromatography may be employed to produce purified PPF polypeptide compositions of the present invention.

Pharmaceutical Compositions

The present invention also relates to pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of at least one PPF polypeptide of the invention, or a pharmaceutically acceptable salt thereof, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in the delivery of the PPF polypeptides. Such compositions may include diluents of various buffer content (*e.g.*, acetate, citrate, tartrate, phosphate, TRIS), pH and ionic strength; additives such as as surfactants and solubilizing agents (*e.g.*, sorbitan monooleate, lecithin, Pluronics, Tween 20 & 80, Polysorbate 20 & 80, propylene glycol, ethanol, PEG-40, sodium dodecyl sulfate), anti-oxidants (*e.g.*, monothioglyercol, ascorbic acid, acetylcysteine, sulfurous acid salts (bisulfise and metabisulfite), preservatives (*e.g.*, phenol, meta-cresol, benzyl alcohol, parabens (methyl, propyl, butyl), benzalkonium chloride, chlorobutanol, thimersol, phenylmercuric salts, (acetate, borate, nitrate), and tonicity/bulking agents (glycerine, sodium chloride, mannitol, sucrose, trehalose, dextrose); incorporation of the material into particulate preparations of polymeric compounds, such as polylactic acid, polyglycolic acid, *etc.*, or in association with liposomes. Such compositions will influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present PYY analog polypeptides. *See, e.g.*, Remington's Pharmaceutical Sciences 1435-712, 18th *ed,* Mack Publishing Co., Easton, Pennsylvania (1990).

In general, the present PPF polypeptides will be useful in the same way that PP, PYY, or NPY is useful in view of their pharmacological properties. One preferred use is to peripherally administer such PPF polypeptides for the treatment or prevention of metabolic conditions and disorders. In particular, the compounds of the invention

possess activity as agents to reduce nutrient availability, reduce of food intake, and effect weight loss.

The present PPF polypeptides may be formulated for peripheral administration, including formulation for injection, oral administration, nasal administration, pulmonary administration, topical administration, or other types of administration as one skilled in the art will recognize. More particularly, administration of the pharmaceutical compositions according to the present invention may be via any common route so long as the target tissue is available via that route. In a preferred embodiment, the pharmaceutical compositions may be introduced into the subject by any conventional peripheral method, *e.g.*, by intravenous, intradermal, intramusclar, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary (*e.g.*, term release); by oral, sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site. The treatment may consist of a single dose or a plurality of doses over a period of time. Controlled continual release of the compositions of the present invention is also contemplated.

The formulation may be liquid or may be solid, such as lyophilized, for reconstitution. Aqueous compositions of the present invention comprise an effective amount of the PPF polypeptide, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrase "pharmaceutically or pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions. In some cases, it will be convenient to provide a PPF polypeptide and another food-intake-reducing, plasma glucose-lowering or plasma lipid-altering agent, such as an amylin, an amylin agonist analog, a CCK or CCK agonist, or a leptin or leptin agonist, or an exendin or exendin agonist analog, and small molecule cannabinoid CBI receptor antagonists, beta-hydroxysteroid dehydrogenase-1

inhibitors, sibutramine and other drugs marketed for treatment of obesity in a single composition or solution for administration together. In other cases, it may be more advantageous to administer the additional agent separately from said PPF polypeptide.

The PPF polypeptide of the invention may be prepared for administration as solutions of free base, or pharmacologically acceptable salts in water suitably mixed with surface active agents (e.g., sorbitan monooleate, polyoxyethylene sorbitain monolaurate (Tween 20), polyoxyethylene sorbitan monooleate (Tween 80), lecithin, polyoxyethylene-polyoxypropylene copolymers (Pluronics), hydroxypropylcellulose,) or complexation agents (e.g., hydroxypropyl-b-cyclodextrin, sulfobutyether-b-cyclodextrin (Captisol), polyvinylpyrrolidone). Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Such products are readily prepared by procedures well known to those skilled in the art. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

In one embodiment, the pharmaceutical compositions of the present invention are formulated so as to be suitable for parenteral administration, *e.g.,* via injection or infusion. Preferably, the PPF polypeptide is suspended in an aqueous carrier, for example, in an buffer solution at a pH of about 3.0 to about 8.0, preferably at a pH of about 3.5 to about 7.4, about 3.5 to about 6.0, about 3.5 to about 5.0 or about 3.7 to about 4.7. Useful buffers include sodium acetate/acetic acid, sodium lactate/lactic acid, ascorbic acid, sodium citrate-citric acid, sodium bicarbonate/carbonic acid, sodium succinate/succinic acid, Histidine, Sodium benzoate/benzoic acid, and sodium phosphates, and Tris(hydroxymethyl)arninomehane. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the

preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

The pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form should be sterile and should be fluid to the extent that *is* easily syringable. It is also desirable for the PPF polypeptide of the invention to be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., sorbitol, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), dimethylacetamide, cremorphor EL, suitable mixtures thereof, and oils (e.g., soybean, sesame, castor, cottonseed, ethyl oleate, isopropyl myristate, glycofurol, corn). The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, meta-cresol, benzyl alcohol, parabens (methyl, propyl, butyl), chlorobutanol, phenol, phenylmercuric salts (acetate, borate, nitrate), sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include tonicity agents (for example, sugars, sodium chloride). Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption (for example, aluminum monostearate and gelatin).

Sterile injectable solutions may be prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In general, the PPF compounds may be formulated into a stable, safe pharmaceutical composition for administration to a patient. Pharmaceutical formulations contemplated for use in the methods of the invention may comprise approximately 0.01 to 20% (w/v), preferably 0.05 to 10%, of the PPF compound. The PPF compounds may be in an acetate, phosphate, citrate or glutamate buffer allowing a pH of the final composition of about 3.0 to about 7.0 containing carbohydrate or polyhydric alcohol as tonicity modifier and, optionally, approximately 0.005 to 5.0% (w/v) of a preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol. Such a preservative is generally included if the formulated peptide is to be included in a multiple use product.

In a particular embodiment of the present invention, a pharmaceutical formulation of the present invention may contain a range of concentrations of PPF compounds, *e.g.*, between about 0.01% to about 98% w/w, or between about 1 to about 98% w/w, or preferably between 80% and 90% w/w, or preferably between about 0.01% to about 50% w/w, or more preferably between about 10% to about 25% w/w in this embodiment. A sufficient amount of water for injection may be used to obtain the desired concentration of solution. The pharmaceutical formulations described herein may be lyophilized. An exemplary formulation can be 1 mg/niL PPF compound in 10 mM sodium acetate buffer solution, pH 4.2, containing 9.3% sucrose as an osmolality modifier.

Generally, a therapeutically or prophylactically effective amount of the present PPF polypeptides will be determined by the age, weight, and condition or severity of the diseases or metabolic conditions or disorders of the recipient. *See, e.g.,* Remington's Pharmaceutical Sciences 697-773. *See also* Wang and Hanson, Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers, Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988). Typically, a dosage of between about 0.001 μg/kg body weight/day to about 1000 μg/kg body weight/day, may be used, but more or less, as a skilled practitioner will recognize, may be used. Dosing may be one, two, three, four or more times daily, or less frequently, such as once a week, once a month, or once a quarter, depending on the formulation, and may be in conjunction with other compositions as described herein.

It should be noted that the present invention is not limited to the dosages recited herein.

Appropriate dosages may be ascertained through the use of established assays for determining level of metabolic conditions or disorders in conjunction with relevant dose-response data. The final dosage regimen will be determined by the attending physician, considering factors that modify the action of drugs, *e.g.*, the drug's specific activity, severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding appropriate dosage levels and duration of treatment for specific diseases and conditions.

An effective dose will typically be in the range of about 0.5 to 30 μg to about 5 mg/day, preferably about 10 to 30 μg to about 2 mg/day and more preferably about 5 to 100 μg to about 1 mg/day, most preferably about 5 μg to about 500 μg/day, administered in a single or divided doses of two, three, four or more administration. Accordingly, exemplary doses can be derived from the total amount of drug to be given a day and the number doses administered a day. For example, exemplary doses can range from about 0.125 μg/dose (0.5 μg given four times a day) to about 2 mg/dose (2 mg given once a day). Other dosages can be between about 0.01 to about 100 μg/kg/dose. The exact dose to be administered may be determined by one of skill in the art and is dependent upon the potency of the particular compound, as well as upon the age, weight and condition of the individual. Administration should begin whenever the suppression of nutrient availability, food intake, weight, blood glucose or plasma lipid lowering is desired, for example, at the first sign of symptoms or shortly after diagnosis of obesity, diabetes mellitus, or insulin-resistance syndrome. Administration may be by any route, *e.g.*, injection, preferably subcutaneous or intramuscular, oral, nasal, transdermal, *etc.* Dosages for certain routes, for example oral administration, may be increased to account for decreased bioavailablity, for example, by about 5-100 fold.

In one embodiment, where the pharmaceutical formulation is to be administered parenterally, the composition is formulation so as to deliver a dose of PPF polypeptide ranging from 0.1 μg/kg to 100 mg/kg body weight/day, preferably at doses ranging

from 1 μg/kg to about 50 nig/kg body weight/day. Exemplary daily amounts may be in the range of a lower limit of 2, 5, 10, 20, 40, 60 or 80 to an upper limit of 80 100, 150, 200, or 250. Parenteral administration may be carried out with an initial bolus followed by continuous infusion to maintain therapeutic circulating levels of drug product. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient.

The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the routes of administration. The optimal pharmaceutical formulation will be determined by one of skill in the art depending on the route of administration and the desired dosage. *See, e.g.,* Remington's Pharmaceutical Sciences, *supra,* pages 1435-1712. Such formulations may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface areas or organ size. Further refinement of the calculations necessary to determine the appropriate treatment dose is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein, as well as the pharmacokinetic data observed in animals or human clinical trials.

It will be appreciated that the pharmaceutical compositions and treatment methods of the invention may be useful in fields of human medicine and veterinary medicine. Thus the subject to be treated may be a mammal, preferably human or other animal. For veterinary purposes, subjects include for example, farm animals including cows, sheep, pigs, horses and goats, companion animals such as dogs and cats, exotic and/or zoo animals, laboratory animals including mice, rats, rabbits, guinea pigs and hamsters; and poultry such as chickens, turkeys, ducks and geese.

In addition, the present invention contemplates a kit comprising a PPF polypeptide of the invention, components suitable for preparing said PPF polypeptide of the invention for pharmaceutical application, and instructions for using said PPF polypeptide and components for pharmaceutical application.

To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLES

The present invention is described in more detail with reference to the following non-limiting examples, which are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof. The examples illustrate the preparation of the present PPF polypeptides, and the testing of these PPF polypeptides of the invention *in vitro* and/or *in vivo*. Those of skill in the art will understand that the techniques described in these examples represent techniques described by the inventors to function well in the practice of the invention, and as such constitute preferred modes for the practice thereof. However, it should be appreciated that those of skill in the art should in light of the present disclosure, appreciate that many changes can be made in the specific methods that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1. Preparation of PPF Polypeptides

Peptides of the invention may be assembled on a Symphony peptide synthesizer (Protein Technologies, Inc.) using Rink amide resin (Novabiochem) with a loading of 0.43-0.49 mmol/g at 0.050-0.100 mmol. Fmoc amino acid (5.0 eq, 0.250-.500 mmol) residues are dissolved at a concentration of 0.10 M in l-methyl-2-pyrrolidinone. All other reagents (HBTU, 1-hydroxybenzotriazole hydrate and N,N-Diisopropylethylamine) are prepared as 0.55 M dimethylformamide solutions. The Fmoc protected amino acids are then coupled to the resin-bound amino acid using, HBTU (2.0 eq, 0.100-0.200 mmol), 1-hydroxybenzotriazole hydrate (1.8 eq, 0.090-0.18 mmol), N,N-diisopropylethylamine (2.4 eq, 0.120-0.240 mmol) for 2 hours. Following the last amino acid coupling, the peptide is deprotected using 20% (v/v) piperidine in dimethylformamide for 1 hour. Once peptide sequence is complete, the Symphony peptide synthesizer is programmed to cleave the resin. Trifluoroacetic

acid (TFA) cleavage of the peptide from resin is carried out using 93% TFA, 3% phenol, 3% water and 1% triisopropylsilane for 1 hour. The cleaved peptide is precipitated using tert-butyl methyl ether, pelleted by centrifugation and lyophilized. The pellet is re-dissolved in water (10-15 mL), filtered and purified via reverse phase HPLC using a C18 column and an acetonitrile/water gradient containing 0.1% TFA. The resulting peptides are purified to homogeneity by reverse phase HPLC and the purity is confirmed by LCMS.

A general procedure for N-capping the peptides of the invention with fatty acids *(e.g.,* octanoic and stearic acids) is as follows: Peptide on rink amide resin (0.1 mmol) is suspended in NMP (5 mL). In a separate vial, HBTU (0.3 mmol), HOBt (0.3 mmol) is dissolved in DMF (5 mL) followed by the addition of DIEA (0.6 mmol). This solution is added to the resin and this suspension is shaken for 2 hrs. The solvent is filtered and washed thoroughly with NMP (5 mLx4) and CH_2Cl_2 (20 mL), dried and is subjected to the TFA cleavage for 1 hr. The yield of the desired peptide is ca. 40 mg after cleavage and purification.

PEG modification may be carried out in solution on a free epsilon-amino group of lysine or a terminal amino group of a purified peptide using commercially available activated PEG esters. The resulting PEGylated derivatives are purified to homogeneity by reverse phase HPLC and the purity is confirmed by LC/MS and MALDI-MS.

The PPF polypeptides of the invention may be tested in a variety of Y-receptor binding assays using binding assay methodologies generally known to those skilled in the art. Such assays include those described below.

NPY Y1 receptor binding assay: Membranes are prepared from confluent cultures of SK-N-MC cells that endogenously expresses the neuropeptide Y1 receptors. Membranes are incubated with 60 pM [^{125}I]- human Peptide YY (2200 Ci/mmol, PerkinElmer Life Sciences), and with unlabeled PPF polypeptide for 60 minutes at ambient temperature in a 96 well polystyrene plate. The well contents are then harvested onto a 96 well glass fiber plate using a Perkin Elmer plate harvester. Dried glass fiber plates are combined with scintillant and counted on a Perkin Elmer scintillation counter.

NPY Y2 receptor binding assay: Membranes are prepared from confluent cultures of SK-N-BE cells that endogenously expresses the neuropeptide Y2 receptors. Membranes are incubated with 30 pM [^{125}I]- human Peptide YY (2200 Ci/mmol, PerkinElmer Life Sciences), and with unlabeled PPF polypeptide for 60 minutes at ambient temperature in a 96 well polystyrene plate. The well contents are then harvested onto a 96 well glass fiber plate using a Perkin Elmer plate harvester. Dried glass fiber plates are combined with scintillant and counted on a Perkin Elmer scintillation counter.

NPY Y4 receptor binding assay: CHO-Kl cells are transiently transfected with cDNA encoding neuropeptide Y4 gene, and then forty-eight hours later membranes are prepared from confluent cell cultures. Membranes are incubated with 18 pM [^{125}I]- human Pancreatic Polypeptide (2200 Ci/mmol, PerkinElmer Life Sciences), and with unlabeled PPF polypeptide for 60 minutes at ambient temperature in a 96 well polystyrene plate. The well contents are then harvested onto a 96 well glass fiber plate using a Perkin Elmer plate harvester. Dried glass fiber plates are combined with scintillant and counted on a Perkin Elmer scintillation counter.

NPY Y5 receptor binding assay: CHO-Kl cells are transiently transfected with cDNA encoding neuropeptide Y5 gene, and then forty-eight hours later membranes are prepared from confluent cell cultures. Membranes are incubated with 44 pM [^{125}I]- human Peptide YY (2200 Ci/mmol, PerkinElmer Life Sciences), and with unlabeled PPF polypeptide for 60 minutes at ambient temperature in a 96 well polystyrene plate. The well contents are then harvested onto a 96 well glass fiber plate using a Perkin Elmer plate harvester. Dried glass fiber plates are combined with scintillant and counted on a Perkin Elmer scintillation counter.

By way of example, Table 2 demonstrates certain preferred PPF polypeptides of the invention and their activity in various Y-receptor binding assays such as those described above.

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
1			0.21	
2	0.2	0.058	4.5	0.31
3	6.2	0.041	54	0.85

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
4	0.48	0.24	39	0.43
5	>1000	229	>1000	0.59
6	0.42	0.19	0.84	0.19
7	1000	21	1000	1000
8	1000	12	1000	1000
9	0.61	0.085	51	0.47
10	1.3	0.023	107	0.49
11	2.6	0.059	96	0.41
12	1.7	0.14	16	0.31
13	3.2	0.42	169	0.54
14	1000	1.6	1000	6.8
15	1.6	0.026	52	0.33
16	4.1	0.048	29	0.15
17	11	0.037	104	0.36
18	0.32	0.031	19	0.32
19	5.4	0.036	117	0.73
20	2.9	0.04	93	0.42
21	24	0.31	182	3.3
22	12	0.1	75	7.4
23	13	0.2	54	3.2
26	4.4	0.04	120	0.42
27	7	0.18	104	1.3
28	0.55	0.032	9.2	0.23
29	14	0.46	178	0.95
50	0.86	0.15	14	0.6
51	0.68	0.14	7.7	0.56
52	2.7	0.19	21	0.93
53	2.2	0.084	7.4	0.64
89	4.7	0.11	38	0.99
90	15	0.46	50	7.3
91	9.2	0.35	99	1.9
92	9.8	0.36	107	5

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
93	8.6	0.28	99	5.6
94	1.8	0.048	27	0.54
95	8.2	0.67	101	7.3
96	7.4	0.29	56	6.6
97	8.6	0.19	54	2.9
98	4.4	0.099	49	2.1
99	3.5	0.065	43	0.99
100	5.9	0.28	70	4
101	8.6	0.18	65	3.4
102	7.8	0.09	58	1.8
103	1.8	0.038	22	0.66
104	4.6	0.053	27	0.89
105	4.4	0.3	68	3.3
106	5.4	0.081	37	0.92
107	11	0.27	70	5.1
108	8.8	0.12	51	2.1
110	20	0.81	97	8.7
111	17	0.41	71	10
112	5.6	0.33	76	6.3
113	6.8	0.1	37	1.2
114	71	0.25	119	14
115	34	6.2	193	55
116	8.9	0.23	40	10
117	7.3	0.21	74	5.8
118	88	0.97	180	31
119	158	1.1	92	47
120	17	1.5	44	27
121	14	0.19	51	14
122	36	0.4	68	2.4
123	45	9.2	66	1.7
124	>1000	86	>1000	56
125	28	9.1	129	8.4
126	24	34	88	2.4

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
127	>1000	>1000	>1000	>1000
128	>1000	113	>1000	>1000
142	6.2	0.12	61	1.2
143	3.8	0.19	56	2.3
144	4.5	0.39	52	4.6
145	5.4	0.12	47.5	1.5
146	8.7	0.19	73	2.3
147	5.1	0.092	48	1.7
150	276	11	1000	118
151	7.6	0.25	115	2.1
152	3.7	0.24	3.9	0.82
157	5.8	0.11	63	1.6
158	6.1	0.11	66	2.1
160	6.3	0.56	71	2.9
162	11	0.47	86	2.8
165	4.8	0.072	59	1.3
170	0.7	0.084	17	0.82
171	33	0.53	97	10
174	11	0.35	64	80
175	20	0.72	>1000	>1000
176	7.6	0.84	120	8.5
177	5.8	0.34	46	11
178	7.7	0.29	38	17
179	30	5.4	33	208
180	4.3	0.11	49	3.9
181	6.3	0.41	46	2.4
182	4.4	0.21	65	5.8
183	4.7	0.071	60	9.2
184	26	0.14	54	42
185	3	0.13	38	3.8
186	0.85	0.11	29	2.8
187	1000	62	1000	128
188	1000	102	1000	968

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
189	1000	57	1000	202
190	1000	24	1000	578
193	308	78	331	180
194	32	1.5	89	15
195	15	1.7	146	5.7
196	1000	612	1000	1000
197	1000	46	611	1000
198	10	0.7	88	9.9
199	38	4.1	143	58
200	106	7	426	74
201	27	2.2	99	29
202	36	148	23	80
203	33	4.4	108	78
204	47	1.1	223	37
205	44	1.5	172	18
206	66	15	204	45
207	180	0.69	1000	114
208	228	93	407	568
211	3.7	0.24	50	5.4
212	2.9	0.046	59	0.8
225	6.7	0.15	79	1.8
226	3	0.059	35	0.57
227	1	0.032	38	0.11
228	4.1	0.1	61	1.1
229	8.2	0.23	57	2.7
230	3.4	0.1	45	1.2
231	5.6	0.37	55	9.4
235	8.7	0.65	77	12
236	6.5	0.24	62	4.6
237	2.1	0.11	35	2.8
238	>1000	229	>1000	0.59
239	0.18	0.092	18	0.27

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
240	2.4	0.059	89	0.58
241	4	0.15	61	0.88
242	2.7	0.13	71	1
243	18	0.74	124	7.2
244	11	1.5	88	7.5
245	0.19	0.077	16	0.35
246	3.9	0.11	119	0.7
247	0.38	0.12	25	0.76
248	0.48	0.12	24	0.44
249	0.36	0.11	21	0.34
250	2.2	0.075	73	0.51
251	0.42	0.12	28	0.52
252	2.1	0.074	52	0.64
253	1.3	0.041	34	0.29
258	0.39	0.12	22	0.89
260	0.42	0.16	22	0.74
261	2.9	0.11	71	1
262	1.7	0.087	61	0.91
263	3.2	0.1	141	1.2
264	1.8	0.22	98	0.48
267	0.25	0.1	9.5	0.32
268	0.31	0.14	21	0.57
269	3.8	0.084	77	0.74
270	3.3	0.13	97	1.4
271	0.51	0.094	4.2	0.25
272	0.26	0.1	12	0.27
273	0.32	0.18	21	0.89
274	4.9	0.42	181	1.5
275	0.59	0.099	81	1.5
276	0.68	0.3	8.3	1.3
277	3.4	0.16	150	2.5
278	3.6	0.078	138	1.4
280	2.1	0.38	108	1.6

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
281	2.8	0.1	117	0.67
282	0.55	0.04	18	0.15
283	30	3.4	87	10.6
285	0.67	0.18	16	0.54
286	0.65	0.11	0.75	0.3
287	5.2	0.16	10	1.2
288	1.8	0.35	11	1.1
289			48	0.83
290			187	0.51
291	186	201	9.5	0.71
292	1.4	0.17	0.77	0.32
293	0.82	0.18	0.87	0.48
294	0.94	0.17	0.98	0.51
295	1	0.18	1	0.63
296	2.7	0.76	2.9	2.1
297	3.6	0.32	4	1.8
298	5.5	1.2	3.4	3.9
299	11	3.2	16	7.5
300	83	16	311	78
301	26	3.7	70	28
302	5.1	0.68	93	2.9
303	6	0.5	7.1	3.3
304	0.51	0.14	0.48	0.28
305				
306	0.6	0.16	1.2	0.27
307	0.53	0.13	0.73	0.47
308	1	0.56	2.1	1.4
309	3.3	78	5.6	1.5
310			27	5.1
311	16	0.49	51	1.8
312			91	3.4
313	9.2	0.57	151	2.6
314	8.2	0.67	202	2.5

SEQ ID NO:	Y1RBA (nM)	Y2RBA (nM)	Y4 RBA (nM)	Y5RBA (nM)
315	9.2	2.1	467	5.6
316	7.1	0.63	52	1.1
317	4.3	0.097	16	0.69
318	100	1.3	84	1.9
319	35	1.04	77	1.2
320	77	3.1	243	13
321	12	3.7	57	5.6
333	4.8	0.54	37	0.87
334	21	0.45	101	2.4
335	34	0.72	109	3.6
338	8.1	0.68	46	1.1
341	1.8	0.15	11	0.3
342	15	0.62	84	1.4
343	12	0.38	69	1.3
347	35	18	740	51

Example 2. PPF Polypeptides Suppress Food Intake in Food Intake Assay

Female NIH/Swiss mice (8-24 weeks old) are group housed with a 12:12 hour lightdark cycle with lights on at 0600. Water and a standard pelleted mouse chow diet are available ad libitum, except as noted. Animals are fasted starting at approximately 1500 hrs, 1 day prior to experiment. The morning of the experiment, animals are divided into experimental groups. In a typical study, n=4 cages with 3 mice/cage.

At time=0 min, all animals are given an intraperitoneal injection of vehicle or compound in an amount ranging from about 10 nmol/kg to 100 nmol/kg, and immediately given a pre-weighed amount (10-15g) of the standard chow. Food is removed and weighed at 30, 60, and 120 min to determine the amount of food consumed (Morley, Flood et al., Am. J. Physiol. 267: R178-R184, 1994). Food intake is calculated by subtracting the weight of the food remaining after the *e.g.*, 30, 60, 120, 180 and/or 240 minute time point from the weight of the food provided initially at time=0. Significant treatment effects were identified by ANOVA ($p<0.05$). Where

a significant difference exists, test means are compared to the control mean using Dunnett's test (Prism v. 2.01, GraphPad Software Inc., San Diego, California).

Figures 1-4 demonstrate the ability of several preferred PPF polypeptides of the invention to reduce cumulative food intake in the food intake assay described above.

Example 3. PPF Polypeptides Decrease Body Weight Gain in High Fat Fed (Diet-induced-obesity, or DIP) C57BL/6 Mice and High Fat-fed HSD Rats.

Mice: Male C57BL/6 mice (4 weeks old at start of study) are fed high fat (HF, 58% of dietary kcal as fat) or low fat (LF, 11% of dietary kcal as fat) chow. After 4 weeks on chow, each mouse is implanted with an osmotic pump (Alzet # 2002) that subcutaneously delivers a predetermined dose of PPF polypeptide continuously for two weeks. Body weight and food intake are measured weekly (Surwit et al., Metabolism—Clinical and Experimental, 44: 645-51, 1995). Effects of the test compound are expressed as the mean +/- sd of % body weight change (i.e., % change from starting weight) of at least 14 mice per treatment group ($p<0.05$ ANOVA, Dunnett's test, Prism v. 2.01, GraphPad Software Inc., San Diego, California).

Rats: The night before treatment, male Sprague-Dawley® rats (average weight = 415) consuming a high fat diet (45% kCal from fat) were assigned to two treatment groups based on equal 24 hr food intake. On test night, each animal received a single IP injection of Vehicle (10%DMSO) or Compound (1 mg/kg) just prior to lights off (180Oh), and were then placed individually into a DietPro automated feeding cage. Each cage contains a food hopper resting on a scale connected to a computer, and a water bottle. Hourly food intake (in grams) is recorded for the following 24 hours. Animals received injections for six consecutive nights. Body weights were recorded nightly.

Figures 5-6 demonstrate the ability of several PPF polypeptides of the invention to decrease body weight gain in the DIO mouse assay described above. Figure 7 demonstrates that once daily injections resulted in a significant reduction in body weight gain on several nights ($P<.05$) in high fat-fed rats. Further, Figure 8 demonstrates that a PPF polypeptide of the invention exhibits greater efficacy than PYY(3-36) in both the food intake assay and the DIO mouse assay.

Example 4. PPF Polypeptides Reduce Blood Pressure

Male Harlan Sprague Dawley rats housed at 22.8 ± 0.8°C in a 12:12 hour light : dark cycle were used to study the effects of PPF Polypeptides on the circulatory system through the use of telemetry. The experiments were performed during the light cycle. Telemetry allows for real-time hemodynamic readings including arterial blood pressure, heart rate and arterial dP/dt, via an implanted radio transmitter in conscious, non-anesthetized, unrestrained rats. In the present Example, rats were injected with either vehicle, 10 nmol/kg PYY, 10 nmol/kg PYY(3-36) or 10 nmol/kg of several PPF polypeptides by remote intravenous dosing. Remote intravenous dosing was achieved through in-dwelling vascular access ports (Access Technologies (Skokie, IL). The port is secured to the underlying muscle just below the skin between the scapulae. The catheter resides in the jugular vein. Data were collected for up to 60 minutes following injection.

As shown in Figures 9A-B, the effect of compound 1 to increase mean arterial pressure are similar to those of PYY(3-36). Figures 9C-D show that while the effects of compound 3 to increase mean arterial pressure and decrease heart rate are similar to those of PYY(I -36), those effects are blunted with compound 2.

Example 5. Antisecretory Effects of PYY and PYY agonists

Gastric Acid Secretion

Male Harlan Sprague Dawley rats were housed at 22.8 ± 0.8°C in a 12:12 hour light : dark cycle. The experiments were performed during the light cycle. Animals, fed rat chow (Teklad LM 485, Madison, WI), were fasted for approximately 20 hours before experimentation. They were given free access to water until the start of the experiment.

The rats (age 11-16 weeks, body mass 291-365 g) were surgically fitted with gastric fistulae custom made by David Osborne, Department of Biology, UCLA. Overnight fasted rats were weighed and their gastric fistulae were uncapped and attached to flexible Tygon tubing (3/8 x 1/16) into which was fitted a piece of PE205 tubing that would extend into the stomach. Saline was injected through the narrower PE205 tubing and the effluent collected from the Tygon tubing. To ensure proper flow through the fistulae and an empty stomach, the stomach was flushed several times

with ~5 ml of room temperature saline solution until flow was easy and the effluent was clean. Gastric acid secretion was measured at 10min intervals by injecting 5mL of saline (pH 7.0) followed by 3ml of air and collecting the effluent. Three ml of each gastric aspirate were titrated to 7.0 with 0.01 N sodium hydroxide using a pH meter (Beckman model number PHI34 Fullerton, CA). The amount of base required for each titration, corrected to the total volume collected, was used to calculate the moles of acid in each sample.

After a baseline sample was collected, and the recovered volume recorded, the animal was given a subcutaneous injection of 125 μg/kg pentagastrin (Sigma, lot#40K0616) and then 10 min. gastric sampling was continued for a further 2 hours. Forty minutes after pentagastrin injection, when a stable plateau of gastric acid secretion was typically observed, the rats were given a subcutaneous injection of (PYY[3-36]) at a dose per animal of 1, 3, 10, 100μg or saline (n= 3, 2, 4, 4, 6 respectively), (3.45,10.34,34.5, 344.8 μg/kg).

As shown in Figure 10, gastric acid output was expressed as % of pentagastrin-stimulated secretion, calculated as the average of time points 20, 30, and 40 minutes after injection of pentagastrin. In response to pentagastrin, gastric acid secretion increased 6.8-fold from a basal rate of 9.3±5.8 μmol/10 min to 62.8±3.8 μmol/10min 40min after injection (grand means: *P<0.01).* PYY(3-36) injected 40 min after pentagastrin dose-dependently and significantly inhibited gastric acid production. With doses of 10μg and 100μg PYY(3-36), acid secretion was reduced by 74.7±7.2% and 84.7±9.7%, respectively (PO.05, PO.01 and PO.01; *t*-test, 20 minutes after PYY(3-36) injection) (see t=60min in Figures 11-17). The dose response for PYY(3-36) inhibition of pentagastrin-stimulated acid secretion is shown in Figure 11. The ED_{50} for the antacid effect of PYY(3-36) was 11.3 1 μg/kg ± 0.054 log units.

Gastric Emptying

To determine the effects of PYY [3-36] on gastric emptying, conscious, non-fasted male Harlan Sprague Dawley rats with vacuum-aspiration lesions of *area postrema* (APx) and sham-operated rats were used. Experiments were performed at least 2 weeks post surgery (weight 426±8 g) and again three weeks later (weight 544±9 g). The rats were randomly assigned to treatment groups. All rats were housed at 22.7° C

in a 12:12h lightrdark cycle (experiments performed during light cycle) and were fed and watered *ad libitum* (diet LM-485 Teklad, Madison, Wisconsin, USA).

PYY[3-36] dissolved in saline was administered as a 0.1 ml subcutaneous bolus in doses of 0, 1 or 10 μg 5 min before gavage of 5μCi D-[3-^{3}H]-glucose (lot #3165036 Dupont, Wilmington, DE, USA) in 1.ml water. The vehicle or different doses of PYY was given s.c. after animals had been given an oral liquid meal.

There were 15 Treatment Groups:

(I) Control saline	n=4
(2) Control 3 μg/kg	n=3
(3) Control 30μg/kg	n=4
(4) Control 90μg/kg	n=5
(5) Control 300μg/kg	n=5
(6) Sham saline	n=5
(7) Sham 3μg/kg	n-2
(8) Sham 30μg/kg	n=4
(9) Sham 90μg/kg	n=3
(lO)Sham 300 μg/kg	n=5
(II) APx saline	n=5
(12) APx 3μg/kg	n=3
(13) APx 30μg/kg	n=3
(14) APx 90μg/kg	n=3
(15) APx 300μg/kg	n=5

Blood was collected from anesthetized tails of the rats at -15, 0, 5, 15, 30, 60 and 90 min after gavage for measurements and the plasma separated to measure the plasma glucose-derived tritium (CPM per 1Oμl counted in β-counter). The appearance of tritium in plasma has previously been shown to reflect gastric emptying. The integrated tritium appearance in plasma was calculated using the trapezoidal method as the increment above the levels before the tritium gavage (the area-under-the-curve for 30 minutes).

In unoperated rats, PYY[3-36]dose-dependently inhibited label appearance, (10.5±1.5, 7.26±1.52 and 3.20±1.21 cpm/μL.min for 30 μg/kg, 90 μg/kg, 300 μg/kg

PYY[3-36], respectively; PO.0001 ANOVA; Figure 12). In sham-AP rats, 1Oμg (n=4) and 30μg PYY[3-36] injections (n=3) also delayed appearance of label compared to saline-injected controls (n=5) in dose dependent manner (11.89±3.23, 9.88±2.45, 18.94±3.23 cpm/μL/min, respectively; P<0.05). Maximal effect of PYY in sham animals was less compared to intact non-operated rats with ED_{50} also lower than in non-operated animals (decreases from 43.77 to 10.20 μg/kg PYY [3-36], In APx rats, gastric emptying was slowed compared to that in sham-APx or unoperated rats (9.38±3.25 cpm/μL/min; PO. 05, 0.05), but was not altered by administration of PYY[3-36]. Regression analysis confirmed absence of dose dependency.

Results showed that PYY9 [3-36] potently regulates the rate of gastric emptying in normal Sprague Dawley rats. A dose-dependent inhibition of gastric emptying was observed following the injection of PYY (10, 30 and 100 μg/rat). The 100 μg dose of PYY produced an inhibition of similar magnitude as that caused by amylin (lOμg). AP-lesioned animals had a tendency to delay gastric emptying compared to non-operated and sham operated rats (n.s.). PYY[3-36] administration had no additional effect on gastric emptying rate in the AP-lesioned animals.

Gallbladder Emptying

To determine the effect of PYY [3-36] on gallbladder emptying, eight week old, male NIH Swiss mice were housed at 22.8± 0.8° in a 12:12 lightdark cycle, and allowed *ad libitum* access to a standard rodent diet (Teklad LM 7012, Madison, WI) and water. The mice were food deprived for 3 hours prior to experimentation. At t=0, PYY(3-36), CCK-8 or saline was injected subcutaneously in conscious mice. Thirty min later, mice were euthanized by cervical dislocation, a midline laparotomy was performed and the gallbladder was excised and weighed.

Treatment Groups:

Group A: saline lOOμl subcutaneously att=O, n=14.

Group B: PYY(3-36) 1μg/kg subcutaneously at t=0, n=6.

Group C: PYY(3-36) 1Oμg/kg subcutaneously at t=0, n=10.

Group D: PYY(3-36) lOOμg/kg subcutaneously at t=0, n=8.

Group E: CCK-8 1μg/kg subcutaneously at t=0, n=3.

Group F: CCK-8 10μg/kg subcutaneously at t=0, n=3.

Group G: PYY(3-36) 10μg/kg + CCK-8 1 μg/kg subcutaneously at t=0, n=4.

Group H: PYY(3-36) 10μg/kg + CCK-8 10μg/kg subcutaneously at 1=0, n=4.

The results are shown in Figures 18 and 19. PYY(3-36) dose dependently inhibited basal gallbladder emptying with an ED_{50} of 9.94 μg/kg+0.24 log units. The highest dose (Group D) increased gallbladder weight by 168% over that observed in saline injected controls (Group A) (P< 0.005). PYY(3-36) did not affect CCK-8 stimulated gallbladder emptying. The data indicate that PYY[3-36] inhibits gallbladder emptying via CCK-independent pathways. Gallbladder emptying in response to exogenous CCK was not affected by PYY(3-36). A similar result was obtained with PYY[I -36] in conscious dogs; a 400 ng/kg bolus + 800 pmol/kg/h infusion did not inhibit CCK-8-stimulated gallbladder contraction.

It is possible that the effects of PYY(3-36) on gallbladder emptying are mediated by vagal-cholinergic pathways. This idea is supported by findings that specific peptide YY (PYY) binding sites have recently been autoradiographically identified in the *area postrema,* nucleus of the solitary tract, and dorsal motor nucleus regions (collectively referred to as the dorsal vagal complex (DVC)) in rats. These medullary brain stem regions are responsible for vagovagal reflex control of gastrointestinal functions, including motility and secretion. PYY(3-36) inhibits other digestive functions that are mediated by vagal-cholinergic mechanisms, such as gastric emptying.

Example 6. Gastroprotective effects of PYY and PYY agonists

Male Harlan Sprague Dawley rats were housed at 22.8± 0.8° in a 12:12 lightdark cycle, and allowed *ad libitum* access to a standard rodent diet (Teklad LM 485, Madison, WI) and water. The rats, 200-220 gm, were fasted for approximately 20 hours prior to experimentation.

At t=-30, PYY(3-36) or saline was injected s.c. At t=0, a 1 ml gavage of absolute ethanol (ethyl alcohol-200 proof dehydrated alcohol, U.S.P. punctilious) or saline was administered. At t=30, the rats were anesthetized with 5% isofluorane. A midline laparotomy incision was made. The stomach was exposed and ligated at the pyloric

and lower esophageal sphincters. The stomach was excised, opened along the lesser curvature and everted to expose the mucosa. The mucosa was gently rinsed with saline and assessed for damage (ulcerations, dilated blood vessels, sloughing off of the mucosal lining) by observers blinded to the treatment. Mucosal damage was scored between 0 (no damage) and 5 (100% of stomach covered by hyperemia and ulceration).

Treatment Groups:

Group A: saline 100μl s.c. at t=-30, gavage 1ml H_2O at t=0, n=4.

Group B: saline 100μl s.c. at t=-30, gavage 1ml absolute ethanol at t=0, n=6.

Group C: PYY(3-36) 1μg/kg at MO, gavage 1ml absolute ethanol at t=0, n=5.

Group D: PYY(3-36) 10μg/kg at MO, gavage 1ml absolute ethanol at 1=0, n=4.

Group E: PYY(3-36) 100μg/kg at MO, gavage 1ml absolute ethanol at 1=0, n=5.

Group F: PYY(3-36) 300μg/kg at t=-30, gavage 1ml absolute ethanol at 1=0, n=5.

PYY(3-36) dose dependently reduced the injury score by 27.4+6.4, 29.3+1 1.6 and 53.7+ 7.9% (n=4,5,5, p<0.05 ANOVA) after injection of 10, 100, and 300 μg/kg of PYY(3-36), respectively (Figure 20). PYY [3-36] showed a gastroprotective effect, in rats. Endogenously circulating PYY [3-36] may play a physiologic role in controlling gastric acid secretion and protecting the gastric mucosa.

Certain preferred PPF oplypeptides are shown in the table below, although other polypeptides are envisioned.

ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	A	Q	Y	A	A	D	L	R	P	Y	I	N	M	L	T	R	P	R	Y	
2		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
3				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
4		Y	P	S	K	P	D	N	P	G	E	D	A	P	A	E	D	M	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
5		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	A	Aib	R	Q	R	Y	
6		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
7		Y	P	P	K	P	E	S	P	G	E	N	A	T	P	E	E	L	A	K	Y	I	S	A	D	R	H	Y	I	N	L	V	T	R	Q	R	Y	
8				P	K	P	E	S	P	G	E	N	A	T	P	E	E	L	A	K	Y	I	S	A	D	R	H	Y	I	N	L	V	T	R	Q	R	Y	
9		A	P	P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	A	K	Y	Y	T	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
10				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	A	K	Y	Y	T	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
11				P	K	P	E	N	P	G	E	D	A	P	P	E	E	L	A	K	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
12	A	Y	P	P	K	P	E	S	P	G	D	A	A	S	P	E	E	I	A	Q	Y	F	S	A	L	R	H	Y	I	N	L	V	T	R	Q	R	Y	
13		M	P	P	K	P	D	N	P	S	S	D	A	S	P	E	E	L	S	K	Y	M	L	A	V	R	N	Y	I	N	L	I	T	R	Q	R	Y	
14				P	K	P	D	N	P	S	S	D	A	S	P	E	E	L	S	K	Y	M	L	A	V	R	N	Y	I	N	L	I	T	R	Q	R	Y	
15				P	K	P	D	N	P	G	D	N	A	S	P	E	Q	M	A	R	Y	K	A	A	V	R	H	Y	I	N	L	I	T	R	Q	R	Y	
16				P	K	P	E	N	P	G	D	N	A	S	P	E	E	M	A	K	Y	F	S	A	L	R	H	Y	I	N	L	V	T	R	Q	R	Y	
17				T	K	P	E	N	P	G	N	D	A	S	P	Q	E	M	A	K	Y	M	T	A	L	R	H	Y	V	N	L	I	T	R	Q	R	Y	
18		Y	P	P	K	P	E	N	P	G	E	D	A	S	P	E	E	M	T	K	Y	L	T	A	L	R	H	Y	I	N	L	V	T	R	Q	R	Y	

ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
19				P	K	P	E	N	P	G	E	D	A	S	P	E	E	M	T	K	Y	L	T	A	L	R	H	Y	I	N	L	V	T	R	Q	R	Y	
20				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	K	Y	Y	T	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
21				S	K	P	D	N	P	G	E	D	A	P	A	E	D	M	A	K	Y	Y	T	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
22				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
23				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	N	R	Y	Y	A	A	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
24				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
25				I		P	E	H	P	G	D	D	A	P	A	E	D	V	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
26				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
27				P	K	P	E	H	P	G	D	D	A	P	A	E	D	V	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
28		Y	P	P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	K	Y	Y	A	A	L	R	H	Y	I	N	L	V	T	R	Q	R	Y	
29				A	K	P	E	N	P	G	D	N	A	P	A	E	Q	M	A	K	Y	L	T	A	L	R	A	Y	V	N	L	I	T	R	Q	R	Y	
30	FORMULA I																																					
31		Y	P	I	hK·	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
32		Y	P	I	K	P	A	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
33		Y	P	I	K	P	E	A	P	A	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
34		Y	P	I	K	P	E	A	P	G	A	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
35		Y	P	I	K	P	E	A	P	G	E	A	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
36		Y	P	I	K	P	E	A	P	G	E	D	A	A	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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37		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
38		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	A	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
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43		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	A	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
44		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	A	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
45		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
46		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	A	V	T	R	Q	R	Y	
47		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
48		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
49		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
50		A	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
51		F	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
52		Y	dA	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
53		Y	G	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
54		A	Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y

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55		Y	A	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
56		Y	P	A	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
57		Y	P	I	A	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
58		Y	P	I	K	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
59		Y	P	I	K	P	A	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
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61		Y	P	I	K	P	E	A	P	A	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
62		Y	P	I	K	P	E	A	P	G	A	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
63		Y	P	I	K	P	E	A	P	G	E	A	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
64		Y	P	I	K	P	E	A	P	G	E	D	A	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
65		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
66		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	A	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
67		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	A	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
68		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	A	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
69		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
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71		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	A	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
72		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	A	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y

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74		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	A	L	R	H	Y	L	N	L	V	T	R	Q	R	Y
75		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	A	R	H	Y	L	N	L	V	T	R	Q	R	Y
76		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	H	Y	L	N	L	V	T	R	Q	R	Y
77		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	A	Y	L	N	L	V	T	R	Q	R	Y
78		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	A	L	N	L	V	T	R	Q	R	Y
79		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	A	N	L	V	T	R	Q	R	Y
80		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	A	L	V	T	R	Q	R	Y
81		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	A	V	T	R	Q	R	Y
82		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	A	T	R	Q	R	Y
83		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	A	R	Q	R	Y
84		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	A	Q	R	Y
85		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	A	R	Y
86		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	A	Y
87		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	A
88	FORMULA II																																					
89				A	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
90				I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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91				I	G	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
92				I	dA	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
93				I	K	A	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
94				I	K	P	A	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
95				I	K	P	E	dA	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
96				I	K	P	E	A	A	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
97				I	K	P	E	A	P	A	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
98				I	K	P	E	A	P	G	A	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
99				I	K	P	E	A	P	G	E	A	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
100				I	K	P	E	A	P	G	E	D	dA	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
101				I	K	P	E	A	P	G	E	D	A	A	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
102				I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
103				I	K	P	E	A	P	G	E	D	A	S	P	A	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
104				I	K	P	E	A	P	G	E	D	A	S	P	E	A	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
105				I	K	P	E	A	P	G	E	D	A	S	P	E	E	A	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
106				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
107				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	A	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
108				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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109				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	(NMe)A	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
110				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	A	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
111				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	A	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
112				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	dA	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
113				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	A	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
114				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	A	R	H	Y	L	N	L	V	T	R	Q	R	Y	
115				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	A	H	Y	L	N	L	V	T	R	Q	R	Y	
116				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	K	H	Y	L	N	L	V	T	R	Q	R	Y	
117				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
118				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	A	L	N	L	V	T	R	Q	R	Y	
119				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	A	N	L	V	T	R	Q	R	Y	
120				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	A	L	V	T	R	Q	R	Y	
121				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	A	V	T	R	Q	R	Y	
122				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	A	T	R	Q	R	Y	
123				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	A	R	Q	R	Y	
124				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	A	Q	R	Y	
125				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	K	Q	R	Y	
126				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	A	R	Y	

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127				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	A	Y	
128				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	A	
129				S	K	P	D	N	P	G	E	D	A	P	A	E	D	M	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
130				P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
131			Isocap	P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
132			AC	P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
133				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	(NMe)Y	
134				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	H	
135				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	H	
136				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	W	
137				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	F	
138				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	F	
139				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y(CH2SO3)	
140				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	P(OH)	
141				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	hR	Y	
142				I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
143				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
144				I	K	P	E	A	P	G	E	D	A	S.	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	

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145				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
146				I	K	P	E	A	P	G	E	D	A	S(Ac)	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
147				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S(Ac)	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
148				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
149				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	hR	H	Y	L	N	L	V	T	R	Q	R	Y	
150			FmocSO3H	I	K(FmocSO3H)	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
151		Isocaproyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
152		Fmoc		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
153		Isobutyloxycarbonyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
154		Isopropyloxycarbonyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
155		n-butyloxycarbonyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
156		ethoxycarbonyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
157				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	S	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
158				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	nV	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
159				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
160		Isocapryl		I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
161				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
162		Isocaproyl		I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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163				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
164		Isocaproyl		I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
165				I	K	P	E	A	P	G	E	D	A	S	P	E	E	M	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
166				I	hK	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
167				I	hK	P	E	A	P	G	E	D	A	S	A	E	E	L	A	hR	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
168				L	hK	P	E	A	P	G	E	D	A	hS	A	E	E	L	A	hR	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
169				I	hK	P	E	A	P	G	E	D	A	S	A	Q	E	L	A	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
170				I	KA	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	K	H	Y	L	N	L	V	T	R	Q	R	Y	
171				I	K	P	E	C	P	G	E	D	A	S	P	E	E	C	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
172				L	E	P	V	Y	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
173				L	E	P	V	Y	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
174				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	YY	A	S	L	R	H	Y	L	N	A	V	T	R	Q	R	Y	
175						P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
176											E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
177																E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
178																					Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
179																										R	H	Y	L	N	L	V	T	R	Q	R	Y	
180		Y	P	I	K	Aminocaproyl									P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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181		Y	P	I	K	Aminocaproyl										E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
182		Y	P	I	K	Aminocaproyl											E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
183		Y	P	I	K	Aminocaproyl																	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
184		Y	P	I	K	Aminocaproyl																				R	H	Y	L	N	L	V	T	R	Q	R	Y	
185		Y	P	I	K	P	E	A	P	G	E	A							N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
186		Y	P	I	K	P	E	A	P	G	E	Ado							N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
187			Fmoc	I	K(PEG 5000)	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
188				Fmoc	K(PEG 5000)	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
189			Fmoc	I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K(PEG 5000)	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
190			PEG 5000	I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
191				I	K	P	E	A	P	G	E	D	A	acylation with fatty acids	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
192				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	acylation with fatty acids	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
193			Fmoc	I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K(oct)	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
194				I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K(oct)	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
195			octanoic acid	I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
196			Fmoc	I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K(stea)	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
197				I	A	P	E	A	P	G	E	D	A	S	P	E	E	L	N	K(stea)	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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198										Ac	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
199										stearyl	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
200										octyl	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
201										succinyl	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
202																					stearyl		A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
203																					octyl		A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
204				I	A	P	E	A	P	G	E	D	A	S	P	E	K(PEG)	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
205				I	A	P	E	A	P	G	E	D	A	S	P	E	K(Oct)	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
206			Fmoc	I	A	P	E	A	P	G	E	D	A	S	P	E	K(Oct)	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
207				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y(8-Am-3,6-dioxaoct)	
208				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y(11-Am-undecanoyl)	
209				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y (12 Ado)	
210				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y (8-Oct)	
211				I	K	P	E	A	mimic A		E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
212				I	K	P	E	A	P	mimic A		D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
213				I	K	P	E	A	P	G	mimic A		A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
214				I	K	P	E	A	P	G	E	mimic A		S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
215				I	K	P	E	A	P	G	E	D	mimic A		P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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216				I	K	P	E	A	P	G	E	D	A	mimic A		E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
217				I	K	P	E	A	P	G	E	D	A	S	mimic A		E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
218				I	K	P	E	A	A	Alb	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
219				I	K	P	E	A	P	A	Alb	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
220				I	K	P	E	A	P	G	A	Alb	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
221				I	K	P	E	A	P	G	E	A	Alb	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
222				I	K	P	E	A	P	G	E	D	A	Alb	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
223				I	K	P	E	A	P	G	E	D	A	A	Alb	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
224				I	K	P	E	A	P	G	E	D	A	S	A	Alb	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
225				I	K	P	E	A	A	P	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
226				I	K	P	E	A	P	A	P	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
227				I	K	P	E	A	P	G	A	P	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
228				I	K	P	E	A	P	G	E	A	P	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
229				I	K	P	E	A	P	G	E	D	A	P	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
230				I	K	P	E	A	P	G	E	D	A	S	A	P	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
231				I	K	P	E	A	mimic B		E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
232				I	K	P	E	A	P	mimic B		D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
233				I	K	P	E	A	P	G	mimic B		A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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234				I	K	P	E	A	P	G	E	mimic B		S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
235				I	K	P	E	A	P	G	E	D	mimic B		P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
236				I	K	P	E	A	P	G	E	D	A	mimic B		E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
237				I	K	P	E	A	P	G	E	D	A	S	mimic B		E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
238		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	A	T	R	Q	R	Y	
239		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
240				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
241				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	K	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
242			isocap	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	K	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
243				I		P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
244		Y	P	I		P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
245		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
246				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
247		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
248		Y	P	I	K	P	E	A	P	G	E	D	A	S	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
249		Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
250				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
251		Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	

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252				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
253				P	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
254				P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
255		isocap		P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
256				P	K	P	E	H	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
257				P	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
258		Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
259				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
260		Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
261				I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
262				P	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
263				P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
264			P	P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
265		isocap		P	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
266				P	K	P	E	H	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
267		Y	P	I	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
268		Y	P	I	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
269				I	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	

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270				P	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
271	8-amino octanoyl			I	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
272		Y	P	I	R	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
273		Y	P	I	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
274				I	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
275	P	Y	P	I	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
276	8-amino octanoyl				K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
277				P	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
278				P	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
279		Isocap		P	K	P	E	A	P	G	E	D	A	P	A	E	E	L	A	R	Y	Y	A	S	L	R	A	Y	I	N	L	I	T	R	Q	R	Y	
280	Isocap	Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	A	S	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
281			isocap	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
282			G(Oct)	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
283	Fmoc-G(Oct)			I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
284				G(Oct)	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	R	Y	Y	S	A	L	R	H	Y	I	N	L	I	T	R	Q	R	Y	
285		Y	P	S	K	P	D	N	P	G	E	D	A	P	A	E	D	M	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
286		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
287				L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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288		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	F	L	N	L	V	T	R	Q	R	Y	
289		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	A	P	R	Q	R	Y	
290		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	A	Aib	R	Q	R	Y	
291		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	A	Sar	R	Q	R	Y	
292		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	S	A	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
293		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	K	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
294		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	A	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
295		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
296		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
297		A	P	L	E	P	V	Y	P	G	D	N	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
298		A	P	L	E	P	V	Y	P	G	D	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
299		A	P	L	E	P	V	Y	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
300		A	P	L	E	P	V	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
301		A	P	L	E	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
302		A	P	L	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
303		Isocap A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
304		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
305		Isocap A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	hR	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	

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306		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	hK	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
307		A	P	M	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	H	Y	L	N	L	V	T	R	Q	R	Y	
308		A	P	L	E	P	V	Y	P	G	D	N	A	T	P	E	Q	M	N	R	Y	Y	A	S	L	R	A	Y	L	N	L	V	T	R	Q	R	Y	
309		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	P	R	Y	
310				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	P	R	Y	
311		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
312				I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
313		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	S	L	R	H	Y	I	N	M	L	T	R	Q	R	Y	
314		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	S	A	L	R	H	Y	I	N	M	L	T	R	Q	R	Y	
315		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	H	Y	I	N	M	L	T	R	Q	R	Y	
316		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
317		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	N	Q	Y	A	A	S	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
318		Y	dA	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
319		A	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
320		isocap Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
321		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	hK	R	Y	I	N	M	L	T	R	Q	R	Y	
322		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	hR	R	Y	I	N	M	L	T	R	Q	R	Y	
323		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	Orn	R	Y	I	N	M	L	T	R	Q	R	Y	

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324		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	Cit	R	Y	I	N	M	L	T	R	Q	R	Y	
325		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	hK	Y	I	N	M	L	T	R	Q	R	Y	
326		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	hR	Y	I	N	M	L	T	R	Q	R	Y	
327		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	Orn	Y	I	N	M	L	T	R	Q	R	Y	
328		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	Cit	Y	I	N	M	L	T	R	Q	R	Y	
329		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	hR	hR	Y	I	N	M	L	T	R	Q	R	Y	
330		Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
331		Isocap Y	P	I	hK	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
332		Y	P	I	hR	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
333		Y	P	I	K	P	E	A	hP	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
334		Y	P	I	K	P	E	A	Alb	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
335		Y	P	I	K	P	E	A	G	P	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
336		Y	P	I	K	P	E	A	P	G	E	D	A	S	hP	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
337		Y	P	I	K	P	E	A	P	G	E	D	A	S	Alb	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
338		Y	P	I	K	P	E	A	P	Sar	E	D	A	S	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
339		Y	P	I	K	P	E	A	P	G	E	D	A	hS	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
340		Y	P	I	K	P	E	A	P	G	E	D	A	Dap	P	E	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
341		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	Q	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	

ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
342		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	D	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
343		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	M	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
344		Y	P	I	hK	P	E	A	P	G	E	D	A	S	A	Q	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
345		Y	P	I	hK	P	E	A	P	G	E	D	A	S	A	S	E	L	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
346		Y	P	I	hK	P	E	A	P	G	E	D	A	S	A	Q	E	M	A	Q	Y	A	A	D	L	R	R	Y	I	N	M	L	T	R	Q	R	Y	
347		Y	P	I	K	P	E	A	P	G	E	D	A	S	P	E	E	L	A	Q	Y	A	A	E	L	R	R	Y	I	Q	M	L	T	R	Q	R	Y	
348	Formula III																																					
349	Formula IV																																					
350	Formula V																																					
351	hPYY C-terminal Motif - 32-35																																T	R	Q	R		

While the present invention has been described in terms of preferred examples and embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the.scope of the invention as claimed.

Claims

What is claimed is:

1. A PPF polypeptide comprising an amino acid sequence of Formula (I):

Xaa_1 Xaa_2 Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaag Xaa_{10}
Xaa π Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Xaa_{25} Xaa_{26} Xaa_{27} Xaa_{28} $Xaa_2$9Xaa_3o
Xaa_{31} Thr Arg Gln Arg Xaa_{36}
wherein:
Xaa_1 is Tyr, Ala, Phe, Trp, or absent;
Xaa_2 is Pro, Gly, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is He, Ala, NorVal, Val, Leu, Pro, Ser, Thr or absent;
Xaa_4 is Lys, Ala, Gly, Arg, d-Ala, homoLys, homoArg, Glu, Asp, or absent;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaap is Gly, Ala Ser, sarcosine, Pro, or Aib;
Xaa_1o is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa π is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, Pro, or homoSer;
Xaa_{14} is Pro, Ala, homo-Pro, hydroxyPro, Aib, or Gly;
Xaa_{15} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa_{16} is Glu, Ala, Asp, Asn, or Gln;
Xaa_{17} is Leu, Ala, Met, Trp, He, Val, orNorVal;
Xaa_{18} is Asn, Asp, Ala, Glu, Gln, Ser, or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Asp, Thr, or homoSer;
Xaa_{25} is Arg, homo-Arg, Lys, homoLys, Orn, or Cit;
Xaa_{26} is His, Ala, Arg, homoArg, homoLys, Om, or Cit;
Xaa_{27} is Tyr or Phe;
Xaa_2g is Leu, He, Val, or Ala;
Xaa_{29} is Asn or Gln;
Xaa_{30} is Leu, Ala, NorVal, Val, He, or Met;
Xaa_3] is Ala, Val, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said PPF polypeptide is not a native PPF polypeptide, NPY(2-36), NPY(4-36), PYY(2-36), PYY(4-36), PP(2-36), PP(4-36), Ala^1NPY, Ala^3NPY, Ala^4NPY, Ala^6NPY, Ala^7NPY, Tyr^7pNPY, Ala^9NPY, Ala^{10}NPY, Ala^{11}NPY, Ala^{13}NPY, Gly^{14}NPY,

AIa^{15}NPY, AIa^{16}NPY, AIa^{17}NPY, AIa^{19}NPY, LyS^{19}NPY, AIa^{21}NPY, AIa^{22}NPY, LyS^{25}NPY, AIa^{26}NPY, Phe^{27}NPY, AIa^{28}NPY, GIn^{29}NPY, AIa^{30}NPY, AIa^{31}NPY, Phe^{36}NPY, HIs^{36}NPY, Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Pro^{13}Ala^{14}hPYY, hPP(l-7)-pNPY, hPP(l-17)-pNPY, TyT^{1}NPY, AIa^{7}NPY, or hPP(19-23)-pNPY.

2. The PPF polypeptide of claim 1, with the further proviso that said PPF polypeptide does not include: Phe^{27}hPYY(3-36), Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), Gln^{29}hPYY(3-36), Val^{30}hPYY(3-36), He^{31}IiPYY(S-SO), Leu^{31}hPYY(3-36), Phe^{36}hPYY(3-36), Lys^{25}Phe^{27}hPYY(3-36), Lys^{25}Ile^{28}hPYY(3-36), Lys^{25}VaI^{28}IiPYY(3-36), Lys^{25}Gln^{29}hPYY(3-36), Lys^{25}Val^{30}hPYY(3-36), LyS^{25}IIe^{31}IiPYY(S-SO), Lys^{25}Leu^{31}hPYY(3-36), Lys^{25}Phe^{36}hPYY(3-36), Phe^{27}Ile^{28}hPYY(3-36), Phe^{27}Val^{28}hPYY(3-36), Phe^{27}Gln^{29}hPYY(3-36), Phe^{27}Val^{30}hPYY(3-36), Phe^{27}Ile^{31}hPYY(3-36), Phe^{27}Leu^{31}IiPYY(S-SO), Phe^{27}Phe^{36}hPYY(3-36), Gln^{29}Val^{30}hPYY(3-36), GIn^{29}IIe^{31}IiPYY(S-36), Gln^{29}Leu^{31}hPYY(3-36), Gln^{29}Phe^{36}hPYY(3-36), VaI^{30}IIe^{31}IiPYY(S-SO), VaI$^{3\circ}$Leu^{31}IiPYY(S-S6), or Val^{30}Phe^{36}hPYY(3-36), or Leu^{31}Phe^{36}hPYY(3-36).

3. The PPF polypeptide of claim 1, wherein said PPF polypeptide further comprises one or more insertions.

4. The PPF polypeptide of claim 3, wherein said one or more insertions comprises one or more amino acid insertions.

5. The PPF polypeptide of claim 3, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 54 through SEQ ID NO: 87.

6. The PPF polypeptide of claim 3, wherein said one or more insertions comprises one or more insertions selected from the group consisting of modified amino acids, unnatural amino acids and non-amino acids.

7. The PPF polypeptide of claim 6, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 131 to 132, 150 to 156, 160, 180 to 186, 207 to 210, 219 to 220, 252, 265, 275, 281, 286, 287, 289 to 293, and 313.

8. The PPF polypeptide of claim 1, wherein said polypeptide is linked to one or more water-soluble polymers.

9. The PPF polypeptide of claim 8, wherein said polymer is selected from the group consisting of polyethylene glycol and a fatty acid molecule, wherein said polyethylene glycol and a fatty acid molecule are linked to the N- or C- terminus of the polypeptide, or the side chain of a lysine or serine amino acid residue within the sequence of the polypeptide.

10. The PPF polypeptide of claim 9, wherein said polypeptide is selected from the group consisting of SEQ ID NOs: 187 to 197.

11. The PPF polypeptide of claim 1, wherein said polypeptide comprises an insertion of a β-turn inducing di-peptide selected from the group consisting of Mimic A, Mimic B, Ala-(2-Aminoisobutyric acid) and Ala-Pro.

12. The PPF polypeptide of claim 11, wherein said polypeptide is selected from the group consisting of SEQ ID NOs: 211 to 247.

13. The PPF polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence of Formula II:

Xaai Xaa_2 Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaa_9 Xaa_{10}
Xaaπ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30}
Xaa_{31} Thr Arg Gln Arg Xaa_{36}

wherein:

Xaai is Tyr, Ala, Phe, Trp, or absent;
Xaa_2 is Pro, Gly, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is He, Ala, NorVal, Val, Leu, Pro, Ser or Thr;
Xaa_4 is Lys, Ala, Gly, Arg, d-Ala, homoLys, homoArg, Glu, or Asp;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaag is Gly, Ala Ser, sarcosine, Pro, or Aib;
Xaa_{10} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaaπ is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, or homoSer;
Xaa_{14} is Pro, Ala, homoPro, hydroxyPro, Aib, or Gly;
Xaa_{15} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa_{16} is Glu, Ala, Asp, Asn, or Gln;
Xaa_{17} is Leu, Ala, Met, Trp, He, Val, or NorVal;
$Xaai_8$ is Asn, Asp, Ala, Glu, Gln, Ser or Thr;
Xaajg is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, He, Val, or Ala;
Xaa_{30} is Leu, Ala, NorVal, Val, lie, or Met;
Xaa_{31} is Ala, Val, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, PYY(2-36), PP(2-36), Ala^{13}NPY, Leu^3hPYY(3-36), Val^3hPYY(3-36), hPP(l-7)-pNPY, or hPP(l-17)-pNPY.

14. The PPF polypeptide of claim 13, with the further proviso that said PPF polypeptide does not include: Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), Val^{30}hPYY(3-36), Ile^{31}hPYY(3-36), Leu^{31}hPYY(3-36), Phe^{36}hPYY(3-36), $Val^{30}Ile^{31}$hPYY(3-36), $Val^{30}Leu^{31}$hPYY(3-36), $Val^{30}Phe^{36}$hPYY(3-36), or $Leu^{31}Phe^{36}$hPYY(3-36)

15. The PPF polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence of Formula III:

Xaaj Xaa_2 Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaap Xaa_{10}
Xaa_{11} Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr
Xaa_{21} Xaa_{22} Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30}
Xaa_{31} Thr Arg Gln Arg Xaa_{36}

wherein:

Xaaj is Tyr, Phe, Trp, or absent;
Xaa_2 is Pro, Gly, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa_3 is He, Ala, NorVal, Val, Leu, Pro, Ser or Thr;
Xaa_4 is Lys, Ala, Gly, Arg, d-Ala, homoLys, homoArg, Glu, or Asp;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, or Tyr;
Xaag is Gly, Ala Ser, sarcosine, Pro, or Aib;
Xaa_{10} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaaπ is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
$Xaai_3$ is Ser, Ala, Thr, Pro, or homoSer;
Xaa_{14} is Pro, Ala, homoPro, hydroxyPro, Aib, or Gly;
Xaa_{15} is Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaa_{16} is Glu, Ala, Asp, Asn, or Gln;
Xaa_{17} is Leu, Ala, Met, Trp, He, Val, or NorVal;
Xaa_{18} is Asn, Asp, Ala, Glu, Gln, Ser or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, He, Val, or Ala;
Xaa_3o is Leu, Ala, NorVal, Val, He, or Met;
Xaa_{31} is Ala, Val, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, NPY(2-36), PYY(2-36), PP(2-36), AIa^{3}NPY, AIa^{4}NPY, AIa^{6}NPY, AIa^{7}NPY, Tyr^{7}pNPY, AIa^{9}NPY, AIa^{10}NPY, AIa^{11}NPY, AIa^{13}NPY, GIy^{14}NPY, AIa^{15}NPY, AIa^{16}NPY, AIa^{17}NPY, AIa^{19}NPY, LyS^{19}NPY, AIa^{21}NPY, AIa^{22}NPY$_{5}$ LyS^{25}NPY, AIa^{26}NPY, Phe^{27}NPY, AIa^{28}NPY, GIn^{29}NPY$_{5}$ AIa^{30}NPY, AIa^{31}NPY, Phe^{36}NPY, HiS^{36}NPY, Leu^{3}hPYY(3-36), Val^{3}hPYY(3-36), Lys^{25}hPYY(3-36), Pro^{13}Ala^{14}hPYY, TyT^{1}NPY, AIa^{7}NPY, or hPP(19-23)-pNPY.

16. The PPF polypeptide of claim 15, with the further proviso that said PPF polypeptide does not include: Ile^{2S}hPYY(3-36), Val^{28}hPYY(3-36), Val^{3O}hPYY(3-36), Ile^{31}hPYY(3-36), Leu^{31}hPYY(3-36), Phe^{36}hPYY(3-36), VaI^{30}IIe^{31}IiPYY(S-SO)$_{5}$ Val^{30}Leu^{31}IiPYY(S-SO)$_{5}$ Val^{30}Phe^{36}hPYY(3-36), or Leu^{31}Phe^{36}hPYY(3-36).

17. The PPF polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence of Formula IV:

Xaa$_{1}$ Xaa$_{2}$ Xaa$_{3}$ Xaa$_{4}$ Pro Xaa$_{6}$ Xaa$_{7}$ Pro Xaag Xaa$_{10}$
Xaaπ Xaa$_{12}$ Xaa$_{13}$ Xaa$_{14}$ Xaa$_{15}$ Xaa$_{16}$ Xaa$_{17}$ Xaa$_{18}$ Xaa$_{19}$ Tyr
Xaa$_{21}$ Xaa$_{22}$ Xaa$_{23}$ Leu Arg Xaa$_{26}$ Tyr Xaa$_{28}$ Asn Xaa$_{30}$
Xaa$_{31}$ Thr Arg GIn Arg Xaa$_{36}$

wherein:

Xaa$_{1}$ is Tyr, Phe, Trp, or absent;
Xaa$_{2}$ is Pro, GIy, d-Ala, homoPro, hydroxy-Pro, or absent;
Xaa$_{3}$ is He, Ala, NorVaI, VaI, Leu, Pro, Ser or Thr;
Xaa$_{4}$ is Lys, Ala, GIy, Arg, d-Ala, homoLys, homoArg, GIu, or Asp;
Xaa$_{6}$ is GIu, Ala, VaI$_{5}$Asp, Asn, or GIn;
Xaa$_{7}$ is AIa$_{5}$Asn$_{5}$His, Ser, or Tyr;
Xaag is GIy$_{5}$Ala Ser, sarcosine, Pro, or Aib;
Xaa$_{10}$ is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaat$_{1}$ is Asp, Ala, GIu, Asn, GIn, Pro, Aib, or GIy;
Xaa$_{12}$ is Ala or d-Ala;
Xaa$_{13}$ is Ser, Ala, Thr, or homoSer;
Xaa$_{14}$ is Pro, Ala, homoPro, hydroxyPro, Aib, or GIy;
Xaa$_{15}$ is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaa$_{16}$ is GIu, Ala, Asp, Asn, or GIn;

Xaa_{17} is Leu, Ala, Met, Trp, He, Val, or NorVal;
Xaa_{18} is Asn, Asp, Ala, Glu, Gln, Ser or Thr;
Xaa_{19} is Arg, Tyr, Lys, Ala, Gln, or N(Me)Ala;
Xaa_{21} is Tyr, Ala, Met, Phe, or Leu;
Xaa_{22} is Ala, Ser, Thr, or d-Ala;
Xaa_{23} is Ser, Ala, Thr, or homoSer;
Xaa_{26} is His or Ala;
Xaa_{28} is Leu, lie, Val, or Ala;
Xaa_{30} is Leu, Ala, NorVal, Val, He, or Met;
Xaa_{31} is Ala, Val, He, or Leu; and
Xaa_{36} is Tyr, N(Me)Tyr, His, Trp, or Phe;

with the proviso that said polypeptide is not a native PPF polypeptide, PYY(2-36), Ala^{13}NPY, Leu^{3}hPYY(3-36), or Val^{3}hPYY(3-36)

18. The PPF polypeptide of claim 17, with the further proviso that said PPF polypeptide does not include: Ile^{28}hPYY(3-36), Val^{28}hPYY(3-36), Val^{30}hPYY(3-36), He^{31}IiPYY(S-SO), Leu^{31}IiPYY(S-So), Phe^{36}hPYY(3-36), $Val^{30}Ile^{31}$HPYY(S-SO), $Val^{30}Leu^{31}$hPYY(3-36), $Val^{30}Phe^{36}$hPYY(3-36), or $Leu^{31}Phe^{36}$IiPYY(S-SO)

19. The PPF polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence of Formula V:

Xaa_3 Xaa_4 Pro Xaa_6 Xaa_7 Pro Xaa_9 XaajoXaa π Xaa_{12}
Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Tyr Xaa_2i Xaa_{22}
Xaa_{23} Leu Arg Xaa_{26} Tyr Xaa_{28} Asn Xaa_{30} Xaa_{31} Thr
Arg Gln Arg Xaa_{36}

wherein:

Xaa_3 is He, Ala, Pro, Ser, Thr, or NorVal;
Xaa_4 is Lys, Ala, Gly, Glu, Asp, d-Ala, homoLys, or homoArg;
Xaa_6 is Glu, Ala, Val, Asp, Asn, or Gln;
Xaa_7 is Ala, Asn, His, Ser, Tyr;
Xaa_9 is Gly, Ala, Ser, sarcosine, Pro, or Aib;
Xaa₁ois Glu, Ala, Asp, Asn, Gln, Pro, Aib, or Gly;
Xaaπ is Asp, Ala, Glu, Asn, Gln, Pro, Aib, or Gly;
Xaa_{12} is Ala or d-Ala;
Xaa_{13} is Ser, Ala, Thr, or homoSer;

Xaai$_{4}$ is Pro, Ala, homo-Pro, hydroxyPro, Aib, or GIy;
Xaa$_{15}$ is GIu, Ala, Asp, Asn, GIn, Pro, Aib, or GIy;
Xaa$_{16}$ is GIu, Ala, Asp, Asn, or GIn;
Xaa$_{17}$ is Leu, Ala, Met, Trp, He, VaI, or NorVal;
Xaa$_{18}$ is Asn, Asp, Ala, GIu, GIn, Ser or Thr;
Xaa$_{19}$ is Arg, Tyr, Lys, Ala, GIn, or N(Me)AIa;
Xaa$_{21}$ is Tyr, Ala, Met, Phe, or Leu;
Xaa$_{22}$ is Ala, Ser, Thr, or d-Ala;
Xaa$_{23}$ is Ser, Ala, Thr, or homoSer;
Xaa$_{26}$ is His or Ala;
Xaa$_{28}$ is Leu or Ala;
Xaa$_{3}$o is Leu, Ala, NorVal, or He;
Xaa$_{31}$ is Ala or VaI; and
Xaa$_{36}$ is Tyr, N(Me)Tyr, His, or Trp;

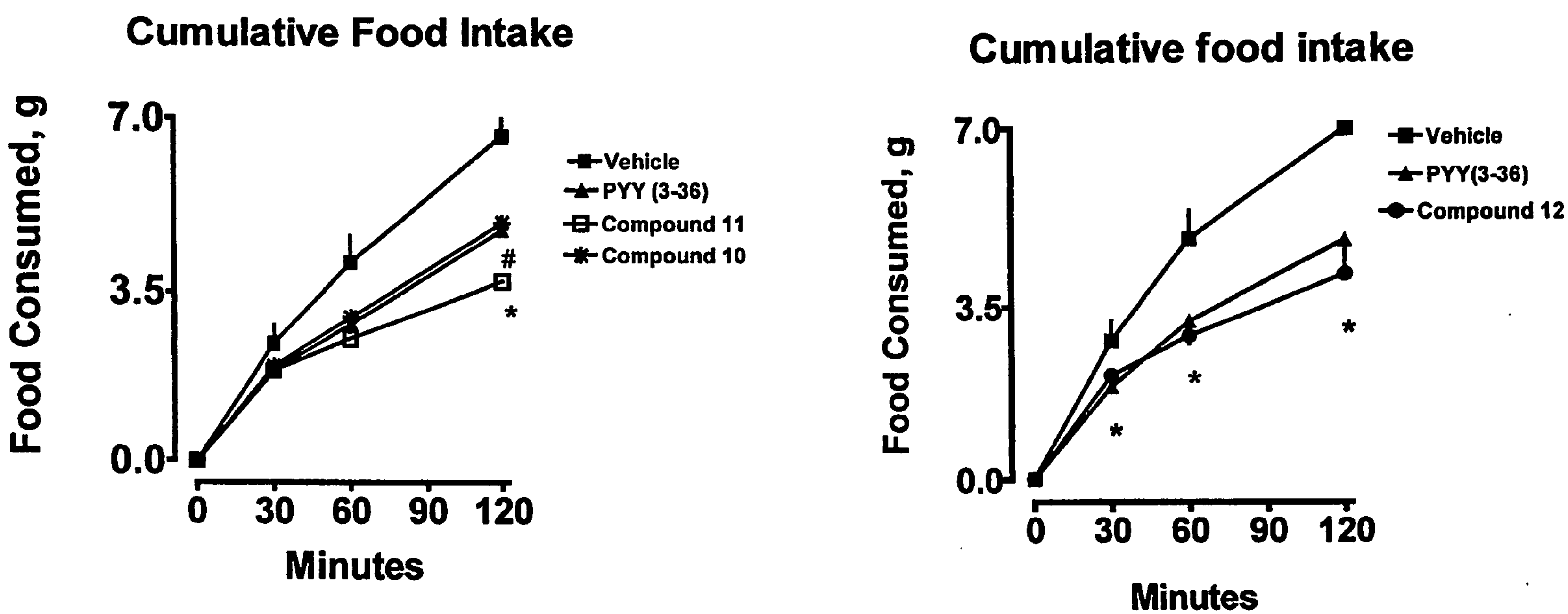
with the proviso that said polypeptide is not a native PPF polypeptide.

20. A PPF polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 93, 95 to 96, 110, 114 to 115, 118, 120, 124 to 129, 139 to 141, 146 to 149, 158, 161 to 164, 167 to 168, 170 to 171, 174 to 186, 198 to SEQ ID NO: 206, 253 to 254, 294, 300 to 301 and SEQ ID NOs: 314 to 317.

21. A PPY polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 to 29.

FIG. 1

Peptides with Combination of Substitutions Are Active in Appetite Suppression



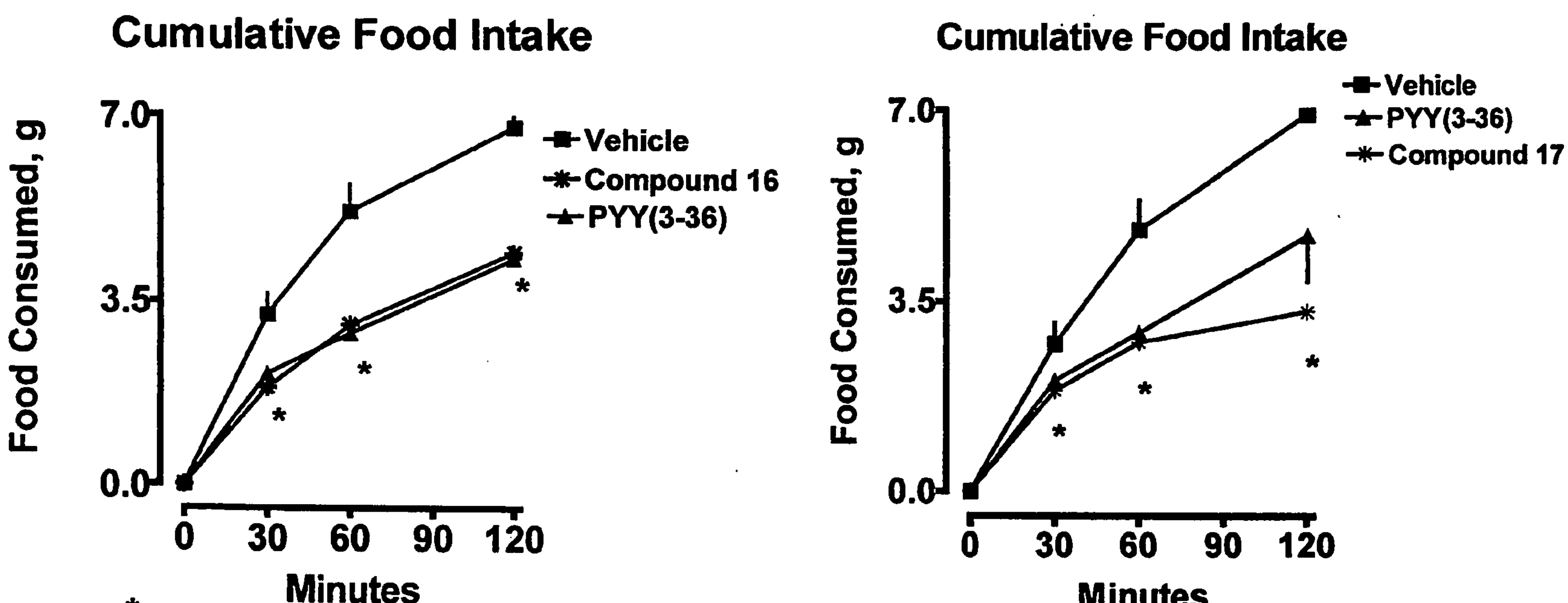
* P<0.01; # P<0.05; ANOVA vs HF vehicle;
N=4 cages; 3 animals/cage

* P<0.01; ANOVA vs HF Vehicle;
N=4 cages; 3 animals/cage

Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 2

PPF Polypeptides with Unnatural AA Substitution or N-Terminus Modification Retain Anorectic Effect

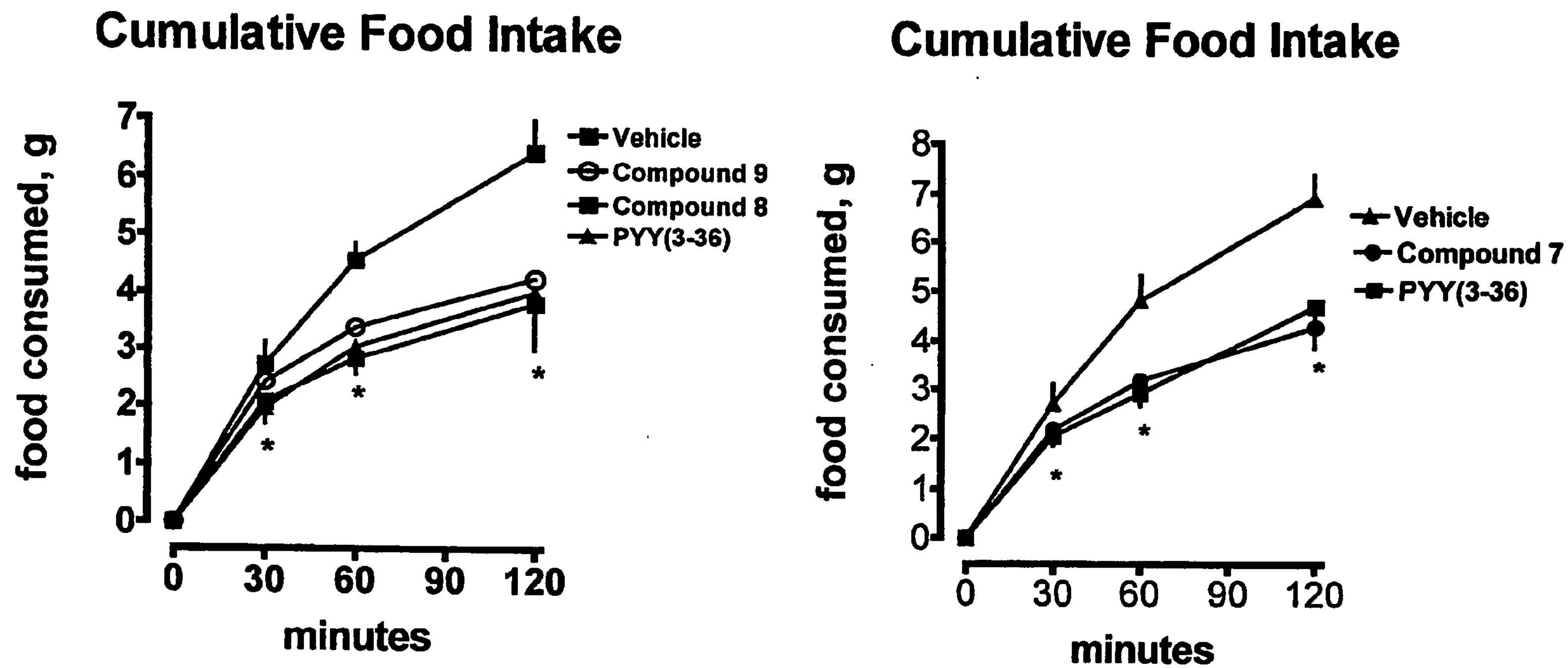


* P<0.01; ANOVA vs HF Vehicle
N = 4 cages; 3 animals/cage

* P<0.01; ANOVA vs HF Vehicle
N = 4 cages; 3 animals/cage

Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 3
PPF Chimeric Polypeptides Are Active in Appetite Suppression



*P<0.01; ANOVA vs HF Vehicle

N = 4 cages; 3 animals/cage

Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 4

PPF Chimeric Polypeptides Are Active in Appetite Suppression

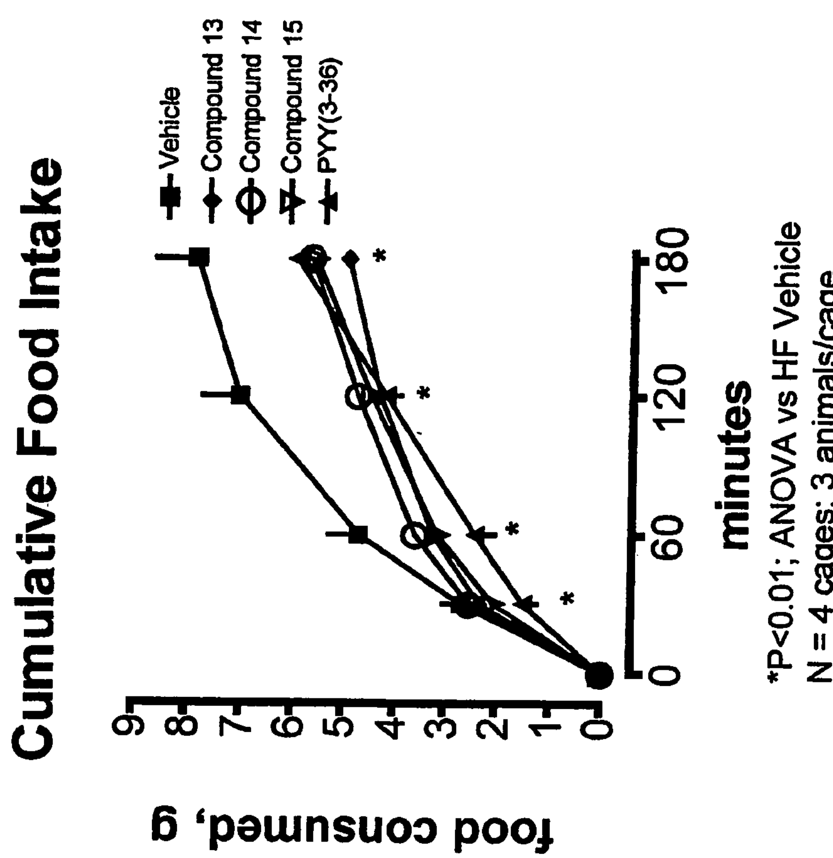
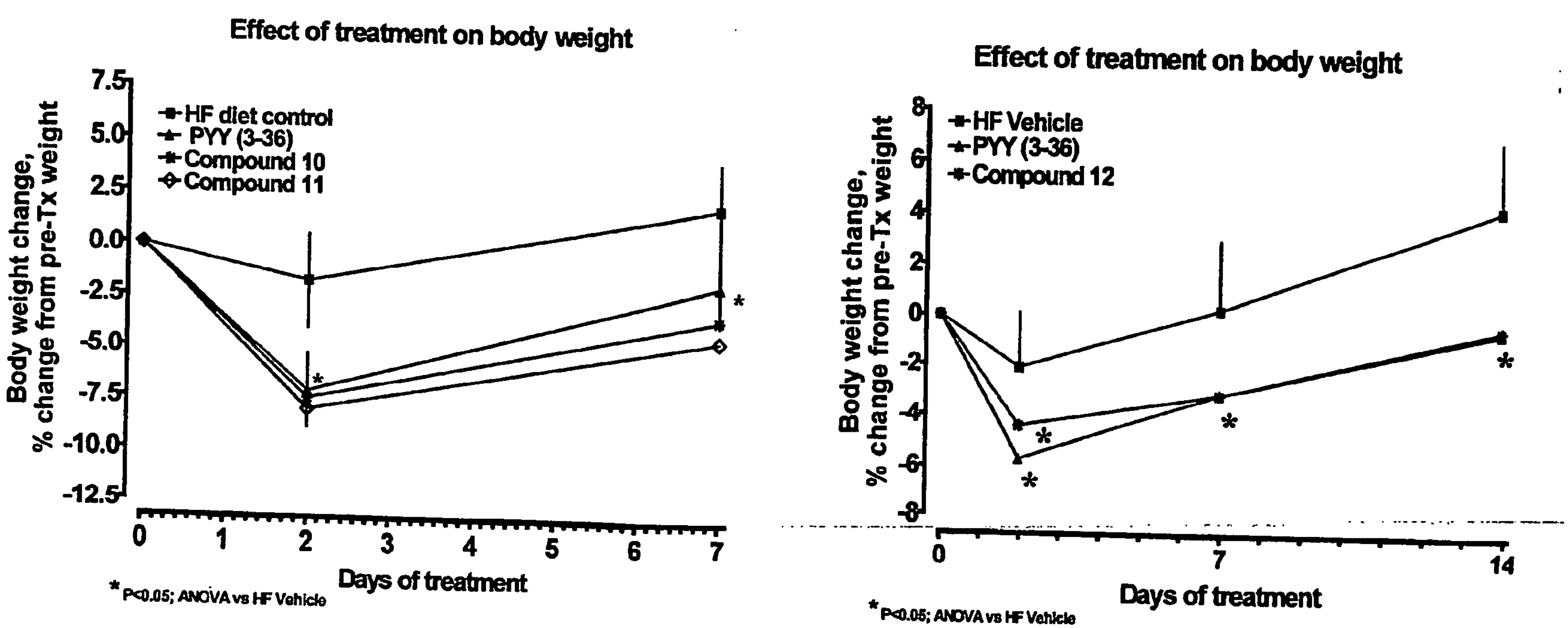


FIG. 5

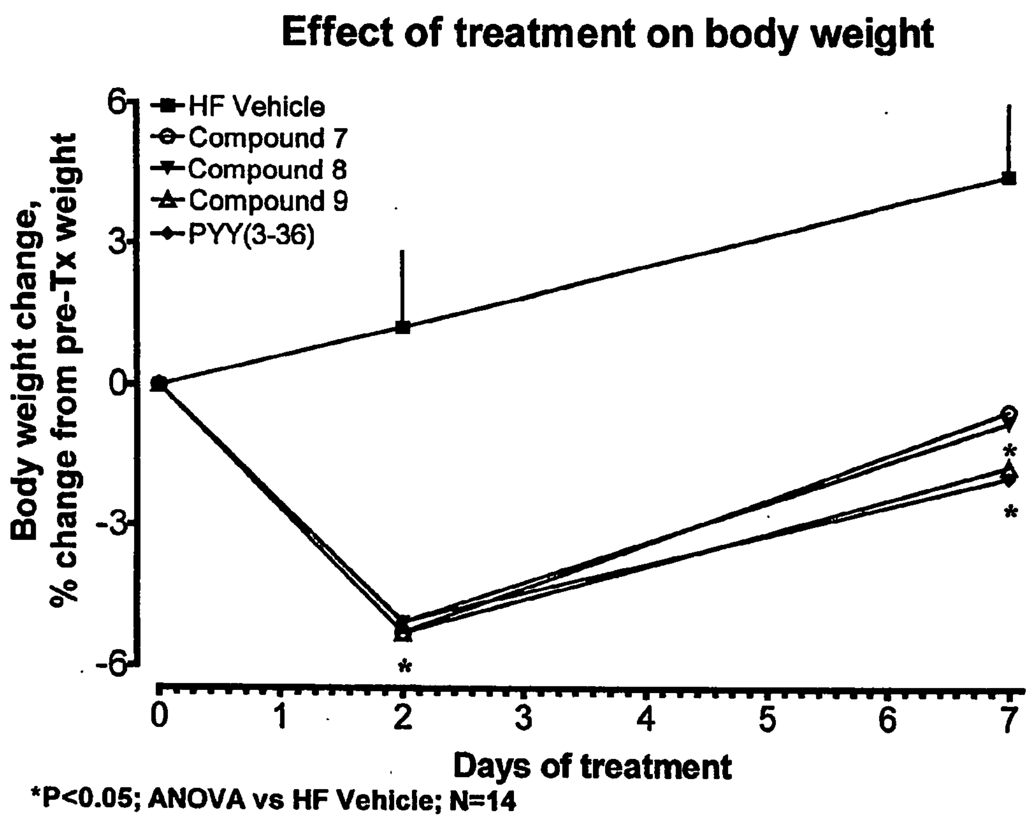
Peptides with Combination of Substitutions Are Active in DIO Mouse Model



DIO Assay, Fattened C57BL/6Mouse Dose: 75 nmol/kg/day, s.c. pump infusion

FIG. 6

PPF Chimeric Polypeptides Are Active in DIO Assay



DIO Assay, Fattened C57BL/6Mouse Dose: 75 nmol/kg/day, s.c. pump infusion

FIG. 7

Weight Gain Following Once Daily IP Injection in Rats: Comparison of PYY [3-36] and PPF Polypeptides

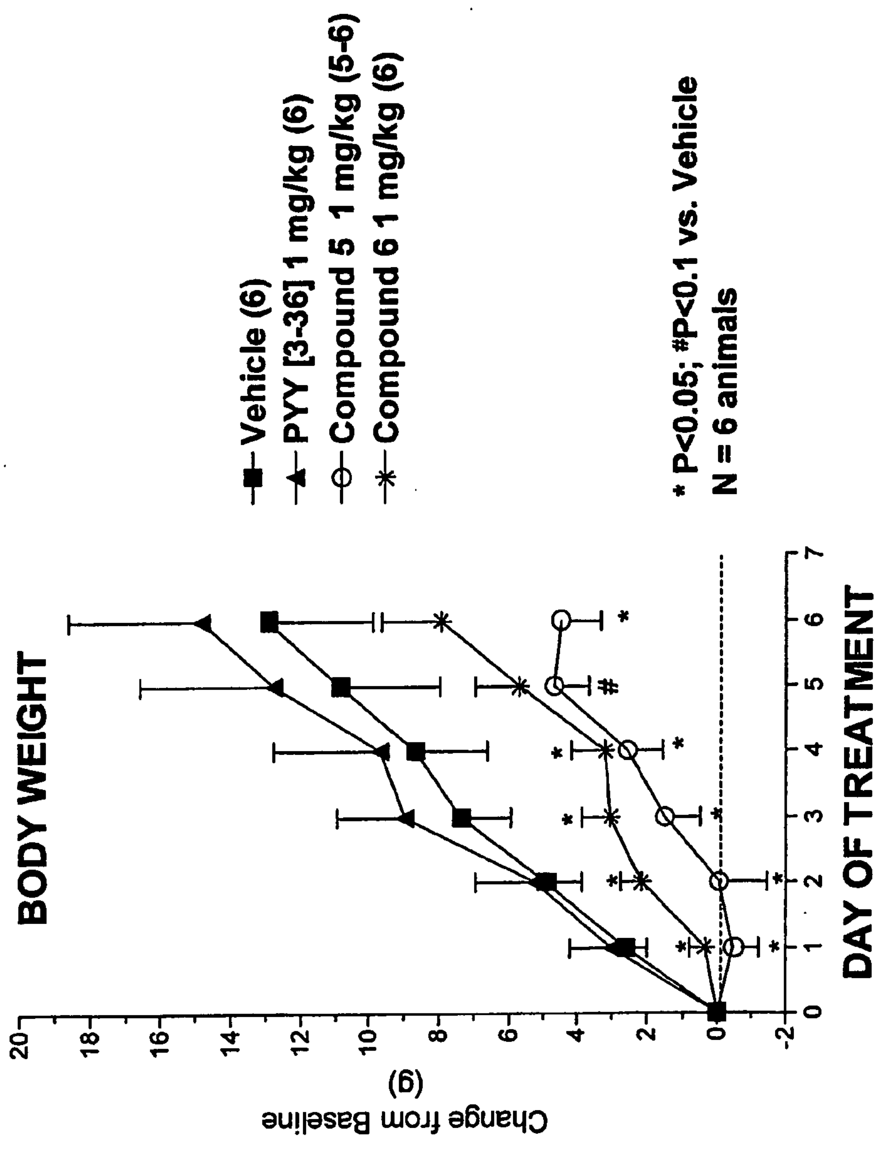
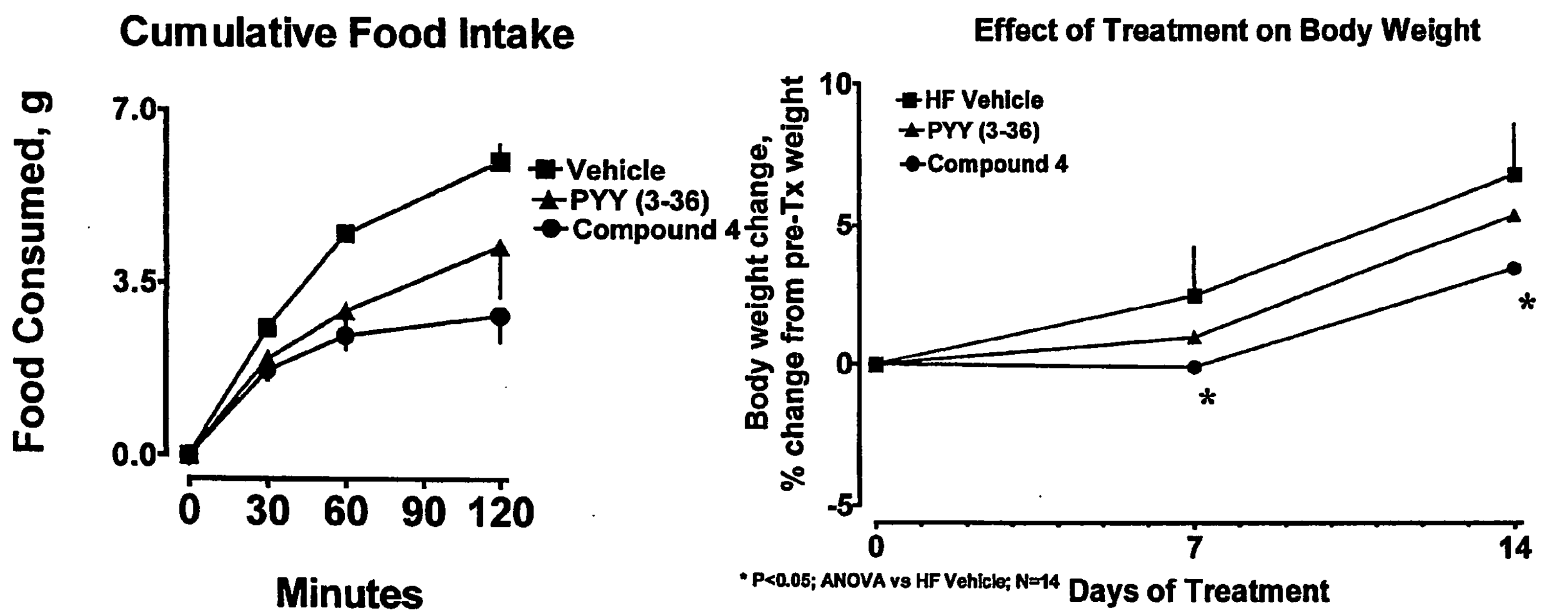


FIG. 8
PPF Polypeptide Shows efficacy in *in-vivo* Assays



Food Intake Assay, Mouse
Dose: 10 nmol/kg i.p.

DIO Assay, Fattened C57BL/6Mouse
Dose: 25 nmol/kg/day s.c. pump infusion

Figure 9-A

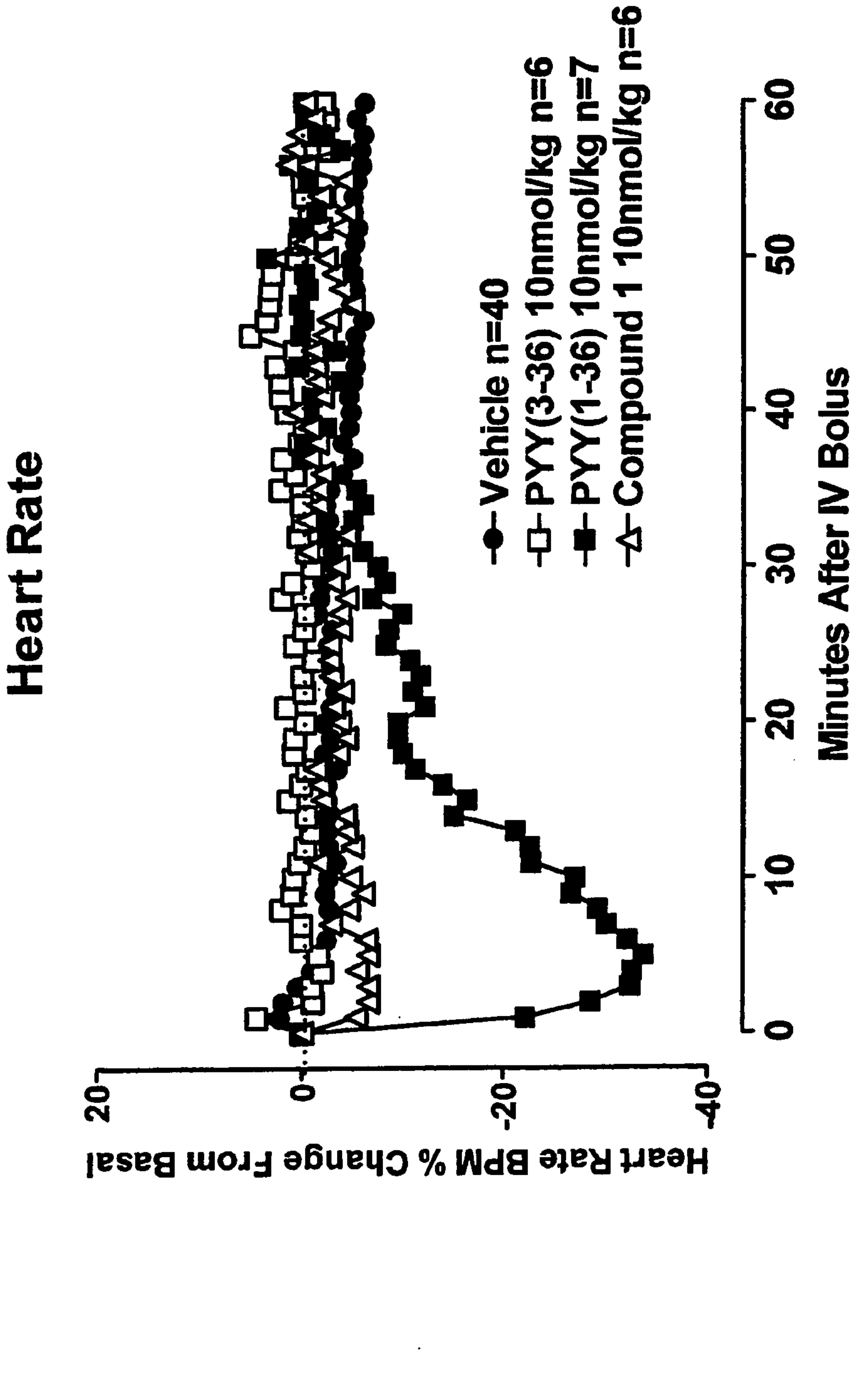

Heart Rate
Heart Rate BPM % Change From Basal
20
0
-20
-40
0
10
20
30
40
50
60
Minutes After IV Bolus
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 1 10nmol/kg n=6

Figure 9-B

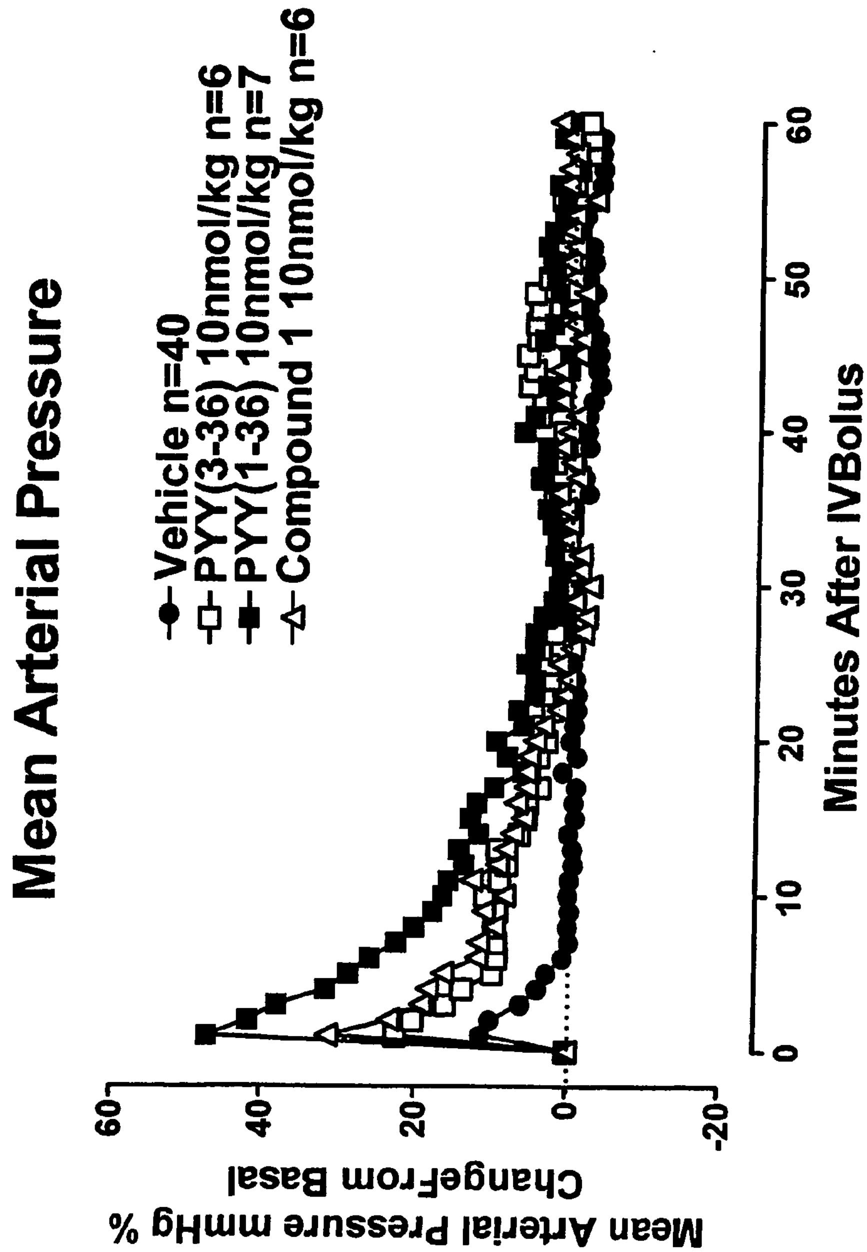

Mean Arterial Pressure
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 1 10nmol/kg n=6
Mean Arterial Pressure mmHg %
ChangeFrom Basal
60
40
20
0
-20
0
10
20
30
40
50
60
Minutes After IVBolus

Figure 9-C

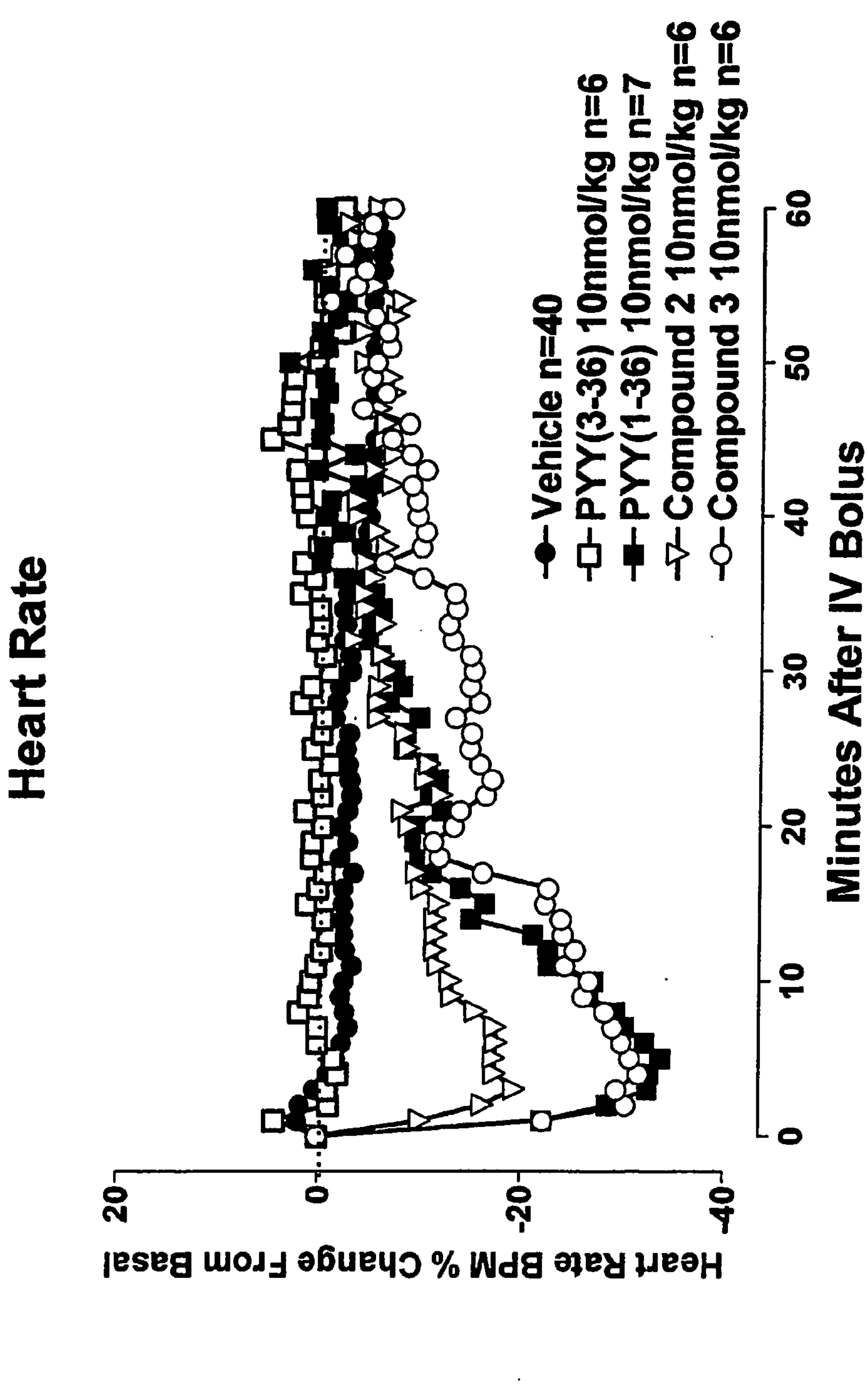

Heart Rate
Heart Rate BPM % Change From Basal
20
0
-20
-40
0
10
20
30
40
50
60
Minutes After IV Bolus
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 2 10nmol/kg n=6
Compound 3 10nmol/kg n=6

Figure 9-D

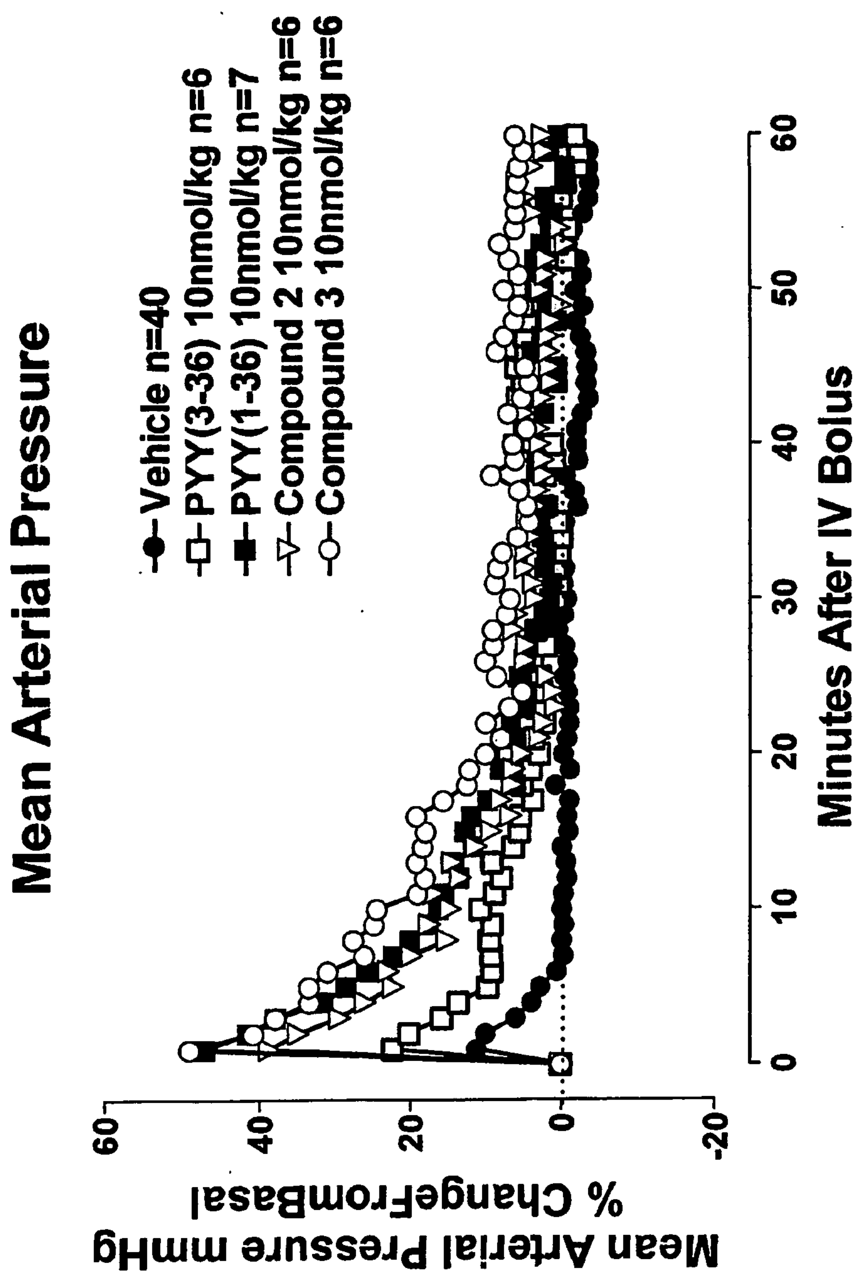

Mean Arterial Pressure
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 2 10nmol/kg n=6
Compound 3 10nmol/kg n=6
Mean Arterial Pressure mmHg
% ChangeFromBasal
60
40
20
0
-20
0
10
20
30
40
50
60
Minutes After IV Bolus

Figure 10

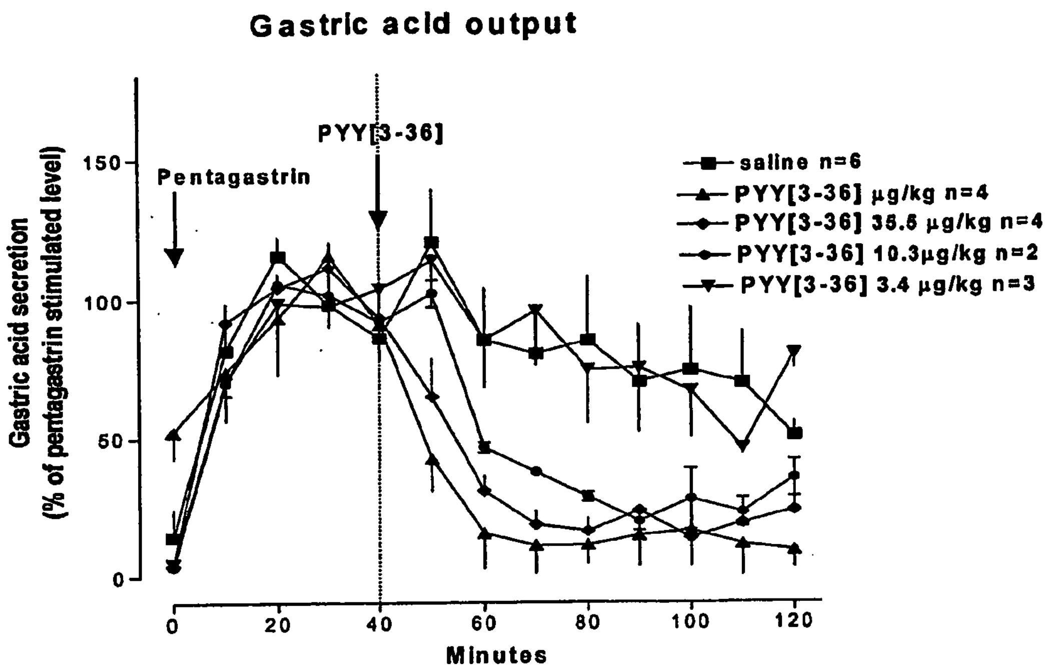

Gastric acid output
Gastric acid secretion
(% of pentagastrin stimulated level)
Minutes
Pentagastrin
PYY[3-36]
saline n=6
PYY[3-36] µg/kg n=4
PYY[3-36] 35.5 µg/kg n=4
PYY[3-36] 10.3µg/kg n=2
PYY[3-36] 3.4 µg/kg n=3
0
50
100
150
0
20
40
60
80
100
120

Figure 11

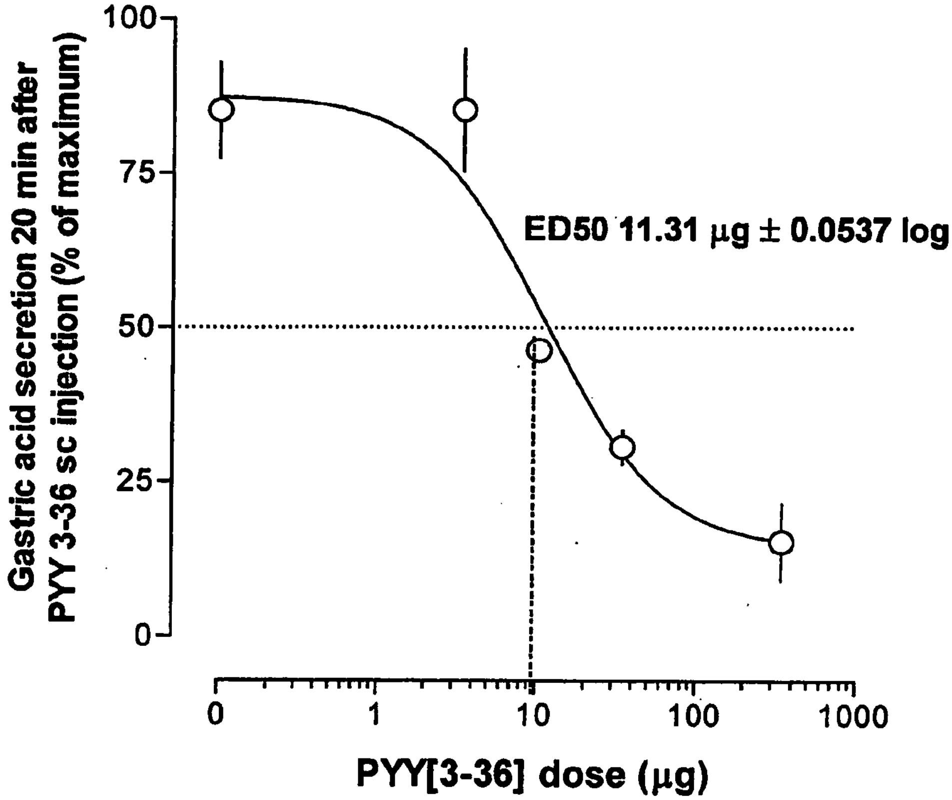

Gastric acid secretion 20 min after
PYY 3-36 sc injection (% of maximum)
100
75
50
25
0
ED50 11.31 µg ± 0.0537 log
0
1
10
100
1000
PYY[3-36] dose (µg)

Figure 12

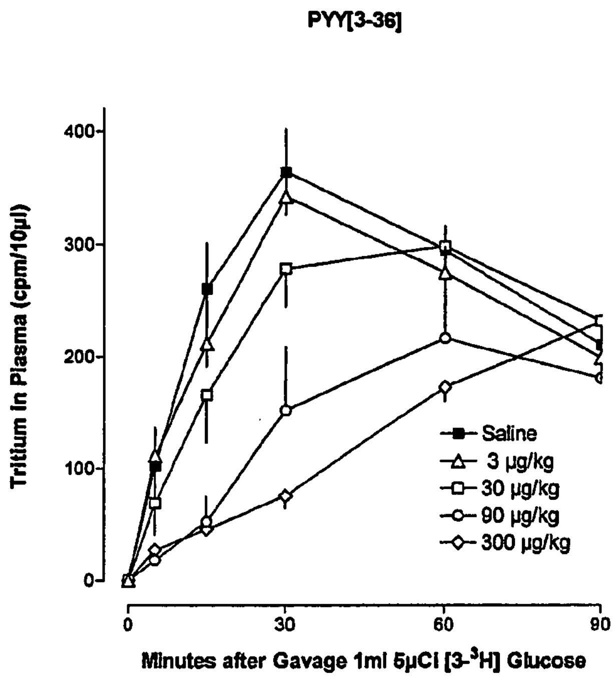
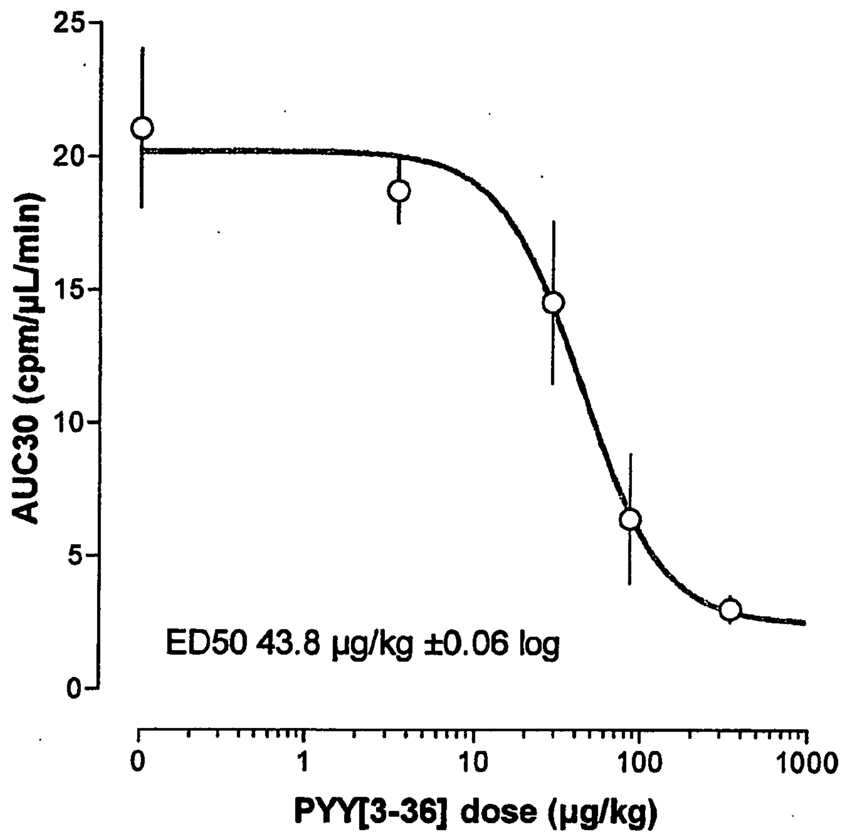

PYY[3-36]
Tritium in Plasma (cpm/10µl)
400
300
200
100
0
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-³H] Glucose
Saline
3 µg/kg
30 µg/kg
90 µg/kg
300 µg/kg

Figure 13



AUC30 (cpm/µL/min)
25
20
15
10
5
0
ED50 43.8 µg/kg ±0.06 log
0
1
10
100
1000
PYY[3-36] dose (µg/kg)

Figure 14

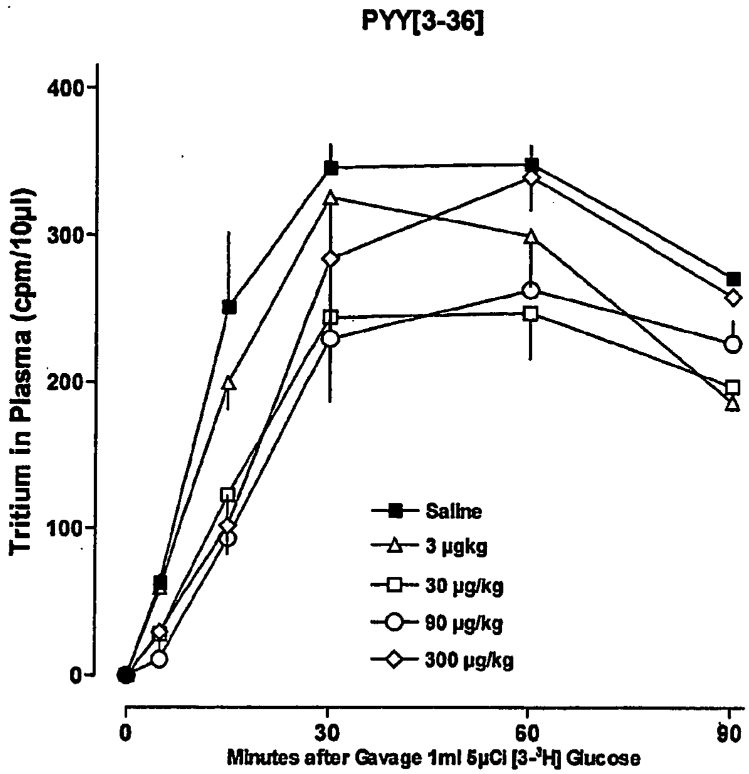

PYY[3-36]
Tritium in Plasma (cpm/10µl)
400
300
200
100
0
Saline
3 µgkg
30 µg/kg
90 µg/kg
300 µg/kg
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-³H] Glucose

Figure 15

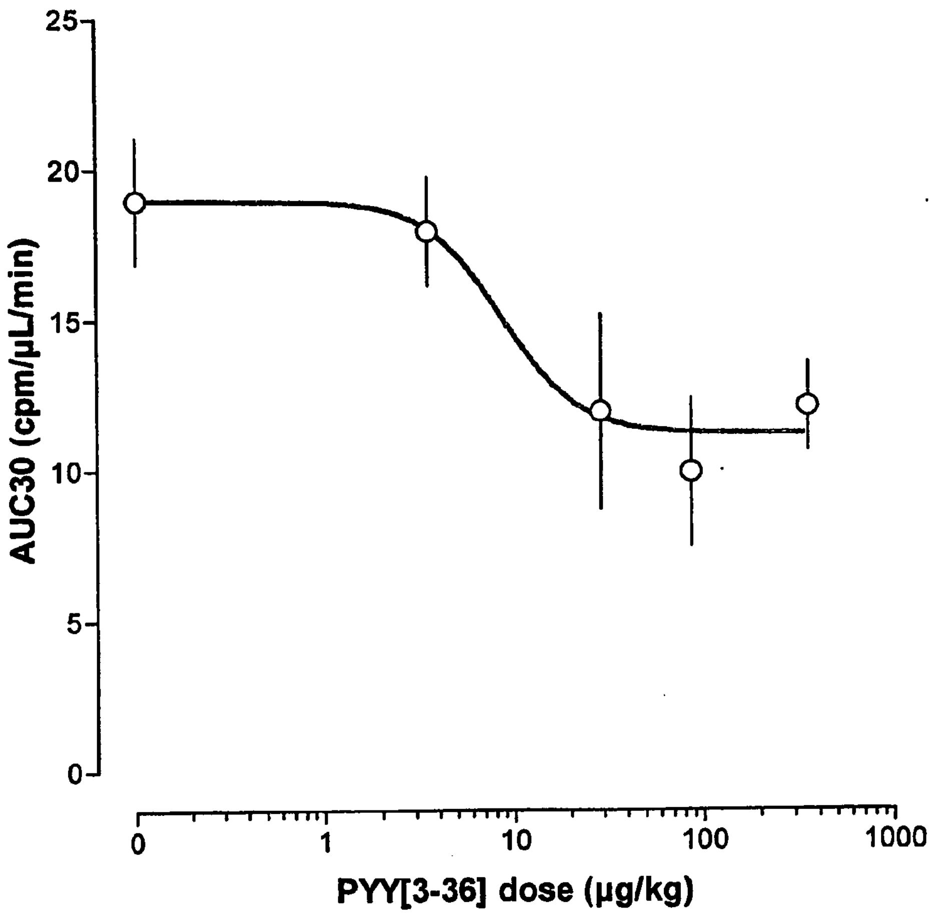


Figure 16

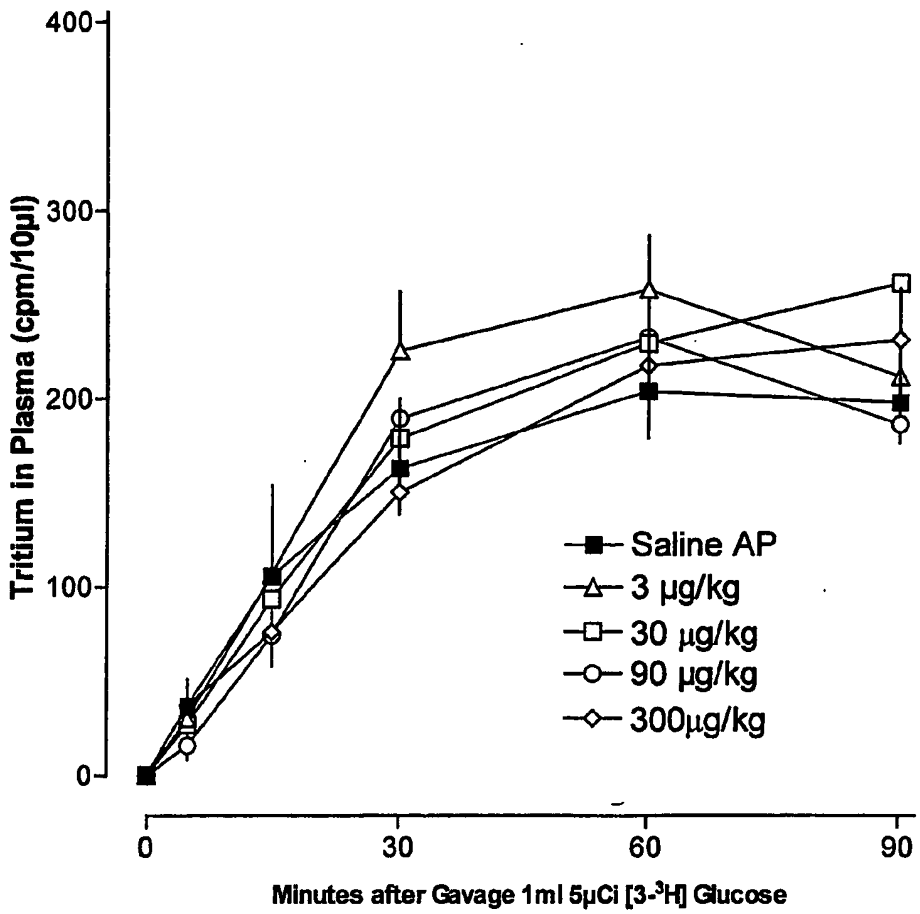

Tritium in Plasma (cpm/10µl)
400
300
200
100
0
Saline AP
3 µg/kg
30 µg/kg
90 µg/kg
300µg/kg
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-³H] Glucose

Figure 17

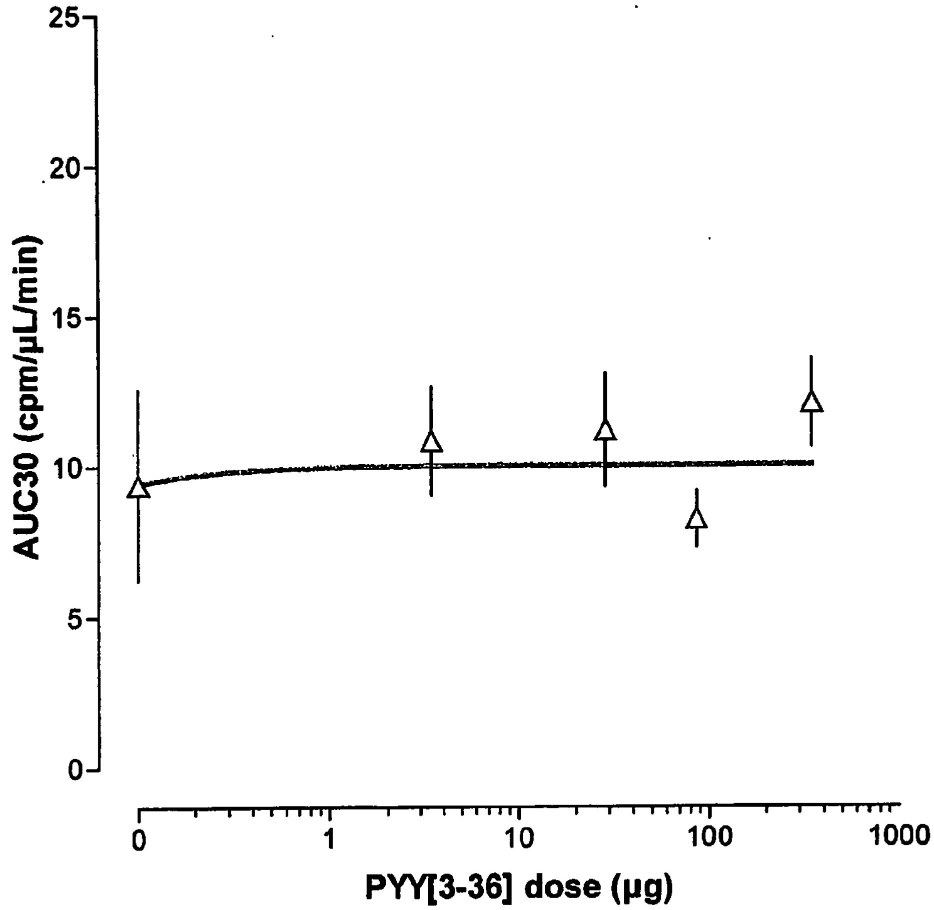
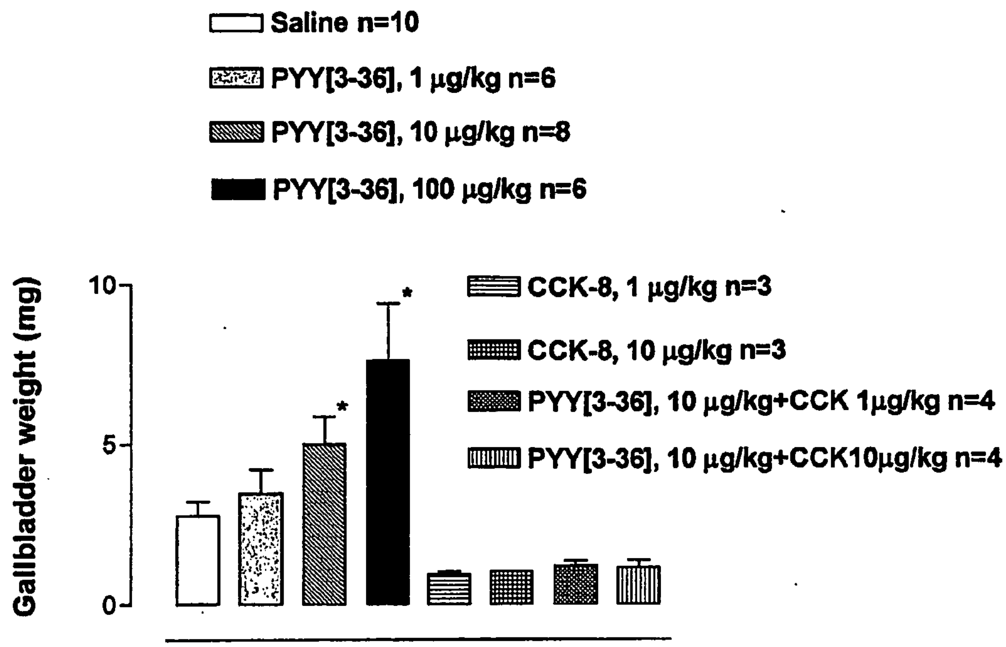
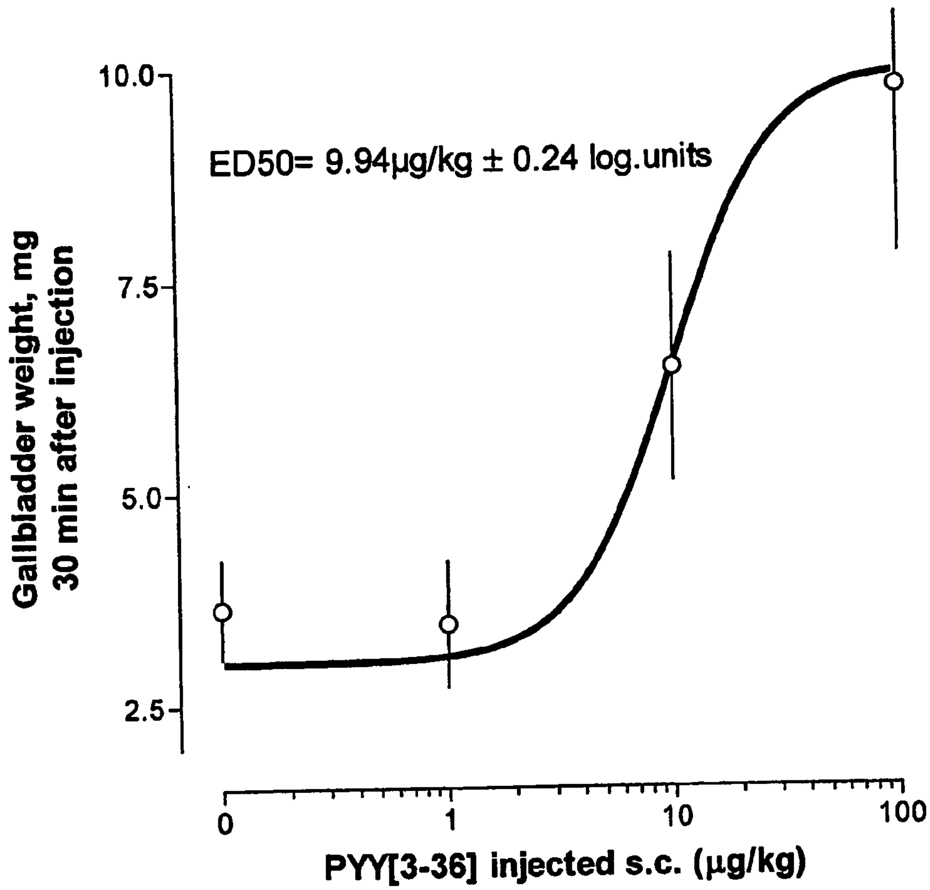


Figure 18



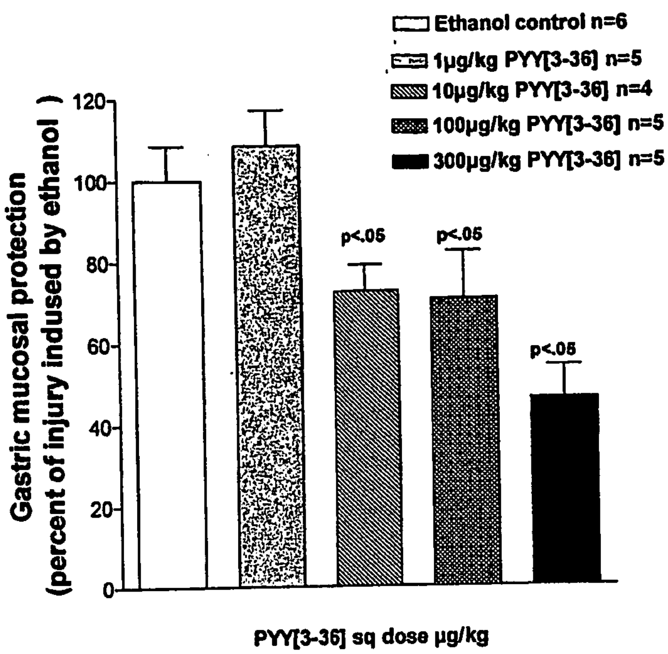
* P< 0.05 compared to saline

Figure 19



10.0
7.5
5.0
2.5
Gallbladder weight, mg
30 min after injection
ED50= 9.94µg/kg ± 0.24 log.units
0
1
10
100
PYY[3-36] injected s.c. (µg/kg)

Figure 20


Ethanol control n=6
1µg/kg PYY[3-36] n=5
10µg/kg PYY[3-36] n=4
100µg/kg PYY[3-36] n=5
300µg/kg PYY[3-36] n=5
Gastric mucosal protection
(percent of injury indused by ethanol)
120
100
80
60
40
20
0
p<.05
p<.05
p<.05
PYY[3-36] sq dose µg/kg